CAMBRIDGE
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THE NEW PHYTOLOGIST

FOUNDED BY A. G. TANSLEY IN 1902

EDITED BY

A. R. CLAPHAM, H. GODWIN, W. O. JAMES

VOLUME XL

PAGES viii AND 338

WITH THREE PLATES AND NUMEROUS FIGURES IN THE TEXT

CAMBRIDGE

AT THE UNIVERSITY PRESS

1941

PRINTED IN GREAT BRITAIN

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ON THE RESPIRATION AND CARBON ASSIMILATION RATES OF SOME ARCTIC PLANTS AS RELATED TO TEMPERATURE

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(With 6 figures in the text)

INTRODUCTION

THE present distribution of plants in the world is controlled by two main groups of factors, namely, those connected with the spread of species from one region to another and those connected with the physiological response of plants to differing environments. The first group of factors includes, among others, such problems as the rate of spread of species, their point and time of origin, mechanical and ecological barriers, while the second group is probably mainly concerned with the temperature and moisture relations of plants.

When an opportunity occurred[1] of doing plant physiological work in Greenland it was thought that the question of possible physiological differences between arctic and temperate or tropical plants should be the central problem to study. It was assumed that temperature is the most important environmental difference between arctic and temperate conditions, since arctic habitats are not usually especially dry. As preliminary physiological studies the effect of temperature on the rate of respiration and of carbon assimilation of a group of plants was investigated, and as an index of the soluble organic matter in plants the osmotic pressure of a group of plants was measured throughout the year (Wager & Wager, 1938*a*). In all these studies the intention was to collect data from as large a group as possible of the species composing the local arctic flora, rather than to work on one or two species intensively, and to use the results for comparison with similar data from plants growing in other climates. In this way it was hoped to make a preliminary analysis of the complex of factors operating in the control of the distribution of arctic plants.

The work was carried out under what were essentially field conditions and no great accuracy is claimed for the individual values recorded, more especially for those of the rates of carbon assimilation, but the experimental errors are probably within the limits of variation that would be shown by different samples of the same species.

[1] The work described in this paper was carried out on the British East Greenland Expedition, 1935–6 under the leadership of L. R. Wager at Kangerdlugssuag, lat. 68° 30′, East Greenland. A general account of the expedition has appeared (L. R. Wager, 1937). The thanks of the writer are due to the Governing Board of Trinity College, Dublin for granting a year's leave of absence to go on the expedition.

Müller (1928), working at Disco, lat. 69°, West Greenland, made a study on two plants, *Chamaenerium latifolium* and *Salix glauca*, of the rate of carbon assimilation as related to light intensity, at temperatures of 10 and 20° C. Consideration of these results will be given with those presented in the present paper.

Kostytschew *et al.* (1930) have worked on the rate of assimilation of arctic plants. A special method has been developed by Kostytschew *et al.* (1928) for determining rates of carbon assimilation, and the results obtained by it with temperate plants do not agree with those obtained using other methods by other workers, either in magnitude or in general character. Since he uses the same method for the work on arctic plants, it is not possible to compare his results with those given in this paper, and all that can be done is to compare the rates found by Kostytschew in temperate regions (1927, 1928) with those obtained by him in the arctic. This comparison shows that the rate of assimilation of arctic plants is somewhat lower than those of temperate plants, at any rate for the four arctic plants investigated by him. This is not in agreement with the results given in this paper or with those of Müller. No respiration rates are given by Kostytschew in these papers on carbon assimilation.

METHODS

A. *Respiration*

The rate of respiration was measured by the output of carbon dioxide which was absorbed in vertical tubes similar to those described by Boysen-Jensen & Müller (1929). During the winter the plants were collected from under snow and allowed to thaw out slowly in the porch of the house, at a temperature near the freezing point. The whole series of readings for any plant species was made on the same lot of material and some of the runs lasted for as long as 15 days, but they were usually only about half this length. The material was healthy in appearance at the end of the experiments.

During the summer, respiration determinations were made in the course of the experiments on carbon assimilation. Plants for these experiments were always gathered fresh each day and were darkened just before starting the respiration determinations. In these cases the values given are the average of readings obtained from more than one batch of material.

B. *Carbon assimilation*

Carbon assimilation was measured by the uptake of carbon dioxide from a stream of normal air in a similar apparatus to that used for the winter respiration experiments. The assimilation chambers, of paraffin wax with a glass cover, were of a suitable size and shape for the leaves of the plant species used. They were 0·3–0·5 cm. deep and had a volume of about 25 c.c. About 3·5–4·0 l. of air were drawn through the chambers during the 20 min. period of a reading. No allowance has been made for the slightly differing carbon-dioxide concentrations in the assimilation chamber due to the different rate of uptake in the determinations at different light intensities as there is no satisfactory way of doing this.

On two occasions the temperature difference between the leaf and the water bath was measured by means of a thermocouple. Under conditions of maximum light intensity (full sunlight) the temperature difference was 3–4° C., but as the light intensity was reduced this rapidly fell to 0·5–0·8° C. No allowance has been made for this, as it would have been impracticable to keep a continuous check on the leaf temperature and the few determinations could not be applied as a uniform correction for all light conditions having the same light intensity. The temperature of the water bath for the values headed "0° C." was 0·3 to 0·6° C., the lowest temperature that could be maintained with ice and water, allowing free space for illuminating the assimilation chambers.

The tank in which the assimilation chambers were placed was outside the hut and the light used was daylight, either full strength or reduced by screens of opal glass or white paper. The intensity of the light was measured by a Weston Photronic cell which had been previously calibrated by Dr H. H. Poole at the Royal Dublin Society. The photo-cell was immersed in the water bath in a suitable container between the two assimilation chambers. The light intensity used is the average of the slightly varying values recorded during the course of the reading. About 15–20 min. were allowed for equilibration to a new light intensity before readings were commenced.

The leaves for the assimilation experiments were collected immediately before use. Stomatal aperture of some of the leaves was tested by the injection method using petrol, and if injection was not complete the batch of leaves was discarded. Wilting of the leaves in the assimilation chamber was common in spite of the air being over-saturated with water. As a result of this, some three-quarters of the runs that were started had to be abandoned, and it was found that many species could not be used at all.

Respiration experiments

Plant species used

The flora of Greenland has been divided by Ostenfeld (1926) into three groups on a basis of its geographical distribution: a "high arctic" or northern group, a "low arctic" or more widespread group and a "southern" group which consists of plants with a southern distribution in Greenland and a temperate distribution outside it. When planning the experiments, it was decided to work with representatives of each of these groups. This was not done, because the common plants round the base hut were of the "low arctic" type and these were the only ones that could be dug up during the winter from under a metre or so of snow. During the summer no runs were done for respiration alone, the suitability of the plants for the assimilation experiments being the deciding factor. In consequence of these two restrictions, only one "high arctic" plant, *Cassiope tetragona*, was used and all the rest were "low arctic" in distribution range. Among the "low arctic" plants used were types with very different ranges of distribution; for instance, *Ranunculus glacialis*, *Diappensia lapponica* and *Saxifraga cernua* have more northern distributions than *Saxifraga oppositifolia*, *Empetrum nigrum* or *Sibbaldia procumbens*, and so

it should be possible even with the present collection of data to distinguish different types of temperature-respiration curves for plants with different geographical distributions, if such exist.

For comparison with the Greenland results experiments were made in Dublin during February and March 1937 on *Empetrum nigrum* and *Saxifraga oppositifolia*, using the same apparatus as had been used in Greenland. The results of these experiments are recorded with those of the arctic plants in Table 1.

RESULTS AND DISCUSSION

In Table 1[1] are given the observed rates of respiration, calculated per gram fresh weight per hour, the Q_{10} calculated as $\frac{R_{T°}+10°}{R_{T°}}$ and the dry weight of one gram fresh weight of the experimental plants. In the experiments there were frequently repeat values of the rate of respiration at given temperatures, and usually the later values were lower than the earlier ones, as, with the exception of *Cassiope hypnoides*, the rate of respiration at a standard temperature fell during the runs, presumably due to starvation effects. The values given in the table are for the first experience that the material had at the stated temperature, and the Q_{10} given is with very few exceptions for the first occasion on which it can be directly calculated. The determinations of rate of respiration at temperatures above 20° C. were made after those at the lower temperatures, and by this time the rate of respiration at 20° C. had fallen, so that the stated rates for respiration at 30 and 40° C. are probably lower than they would have been if they had been the earlier determinations. This should be borne in mind when considering the actual rates of respiration given for these plants. An approximation to the value that would have been obtained if the determination had been made earlier could be obtained by multiplying the 20° C. value by the appropriate Q_{10}.

The Q_{10} values (including those of Müller) have been plotted in Fig. 1 by taking the 0° C. respiration rate as unity and calculating those for other temperatures from this as a base. The curves express only the temperature relationship and not the actual rate of respiration of the material. Since the curves rarely cross each other, their pitch would appear to have significance, at any rate for the sample investigated.

The relative position of the curves does not follow any scheme that can be simply correlated with the distributions of the plants. The curve for *Cassiope tetragona*, the only "high arctic" plant, is not in a unique position; *Cassiope hypnoides*, with a northern type of distribution, gives one of the lowest curves, and the *Empetrum nigrum* and *Saxifraga oppositifolia* investigated in Dublin during the winter give curves in about the same position as the Greenland specimens.

[1] In Table 1 are the data on respiration rate of arctic plants collected by the writer, and for comparison the similar data collected in summer by Müller (1928) and the two sets of values determined in Dublin by the writer. In the subsequent discussion Müller's data will be used with those of the writer. The species are listed in the experimental sequence beginning in October and ending in March.

Table 1. *The respiration rate of some arctic plants, expressed as mgs. of CO_2 per hour per gram fresh weight, at different temperatures and the calculated Q_{10} of respiration for certain temperature ranges*

Plant species	Dry wt. of 1 gm. fresh wt.	Respiration rate at 0°	Q_{0-10}	Respiration rate at 10°	Q_{10-20}	Respiration rate at 20°	Q_{20-30}	Respiration rate at 30°	Q_{30-40}	Respiration rate at 40°
(*a*) Winter determinations:										
Cassiope tetragona shoots	0·51	0·029	**3·3**	0·097	**2·9**	0·28	**2·2**	0·62	**1·7**	0·86
Empetrum nigrum shoots	0·56	0·02	**3·6**	0·083	**2·7**	0·22	**2·2**	0·46	—	—
Cassiope hypnoides shoots	0·36	0·042	**2·1**	0·088	**2·2**	0·19	**2·2**	0·43	**2·1**	0·92
Salix herbacea twigs	—	0·052	**3·5**	0·18	**2·5**	0·46	—	—	—	—
Ranunculus pygmaeus leaves	0·35	0·17	**2·8**	0·32	**3·1**	1·11	**2·1**	1·83	—	—
Antennaria alpina leaves	0·28	0·03	**4·2**	0·119	**3·3**	0·40	**2·0**	0·60	**1·6**	1·23
Diapensia lapponica shoots	0·34	0·04	**3·8**	0·15	**3·4**	0·46	**2·1**	0·72	**2·1**	1·18
Viscaria alpina leaves	0·35	0·08	**2·8**	0·23	**2·2**	0·46	**2·0**	0·82	**2·0**	1·15
Saxifraga oppositifolia shoots	0·46	0·017	**3·0**	0·052	**2·7**	0·14	**2·2**	0·28	**1·9**	0·50
Poa sp. leaves	—	—	**2·7**	—	**2·3**	—	—	—	—	—
(*b*) Summer determinations on leaves:										
Saxifraga cernua	0·09	0·047	**2·3**	0·11	**3·2**	0·35	—	—	—	—
Ranunculus glacialis	0·14	0·10	**3·6**	0·38	**1·4**	0·56	—	—	—	—
Oxyria digyna	0·10	0·10	**3·1**	0·31	**1·6**	0·52	—	—	—	—
Sibbaldia procumbens	0·37	—	—	0·37	**3·2**	1·18	—	—	—	—
Polygonum viviparum	0·21	0·18	**3·0**	0·54	**2·0**	1·06	—	—	—	—
Salix glauca (Müller)	0·28	0·26	**3·2**	0·89	**1·8**	1·53	—	—	—	—
Chamaenerium latifolium (Müller)	0·16	0·13	**2·0**	0·26	**2·5**	0·67	—	—	—	—
Betula nana (Müller)	—	—	—	0·59	**2·2**	1·30	—	—	—	—
(*c*) Determinations in Dublin:										
Empetrum nigrum shoots	0·44	0·019	**3·5**	0·069	**2·7**	0·18	**2·3**	0·39	**2·0**	0·77
Saxifraga oppositifolia shoots	0·24	0·017	**3·9**	0·069	**2·5**	0·17	**2·1**	0·35	**1·8**	0·62

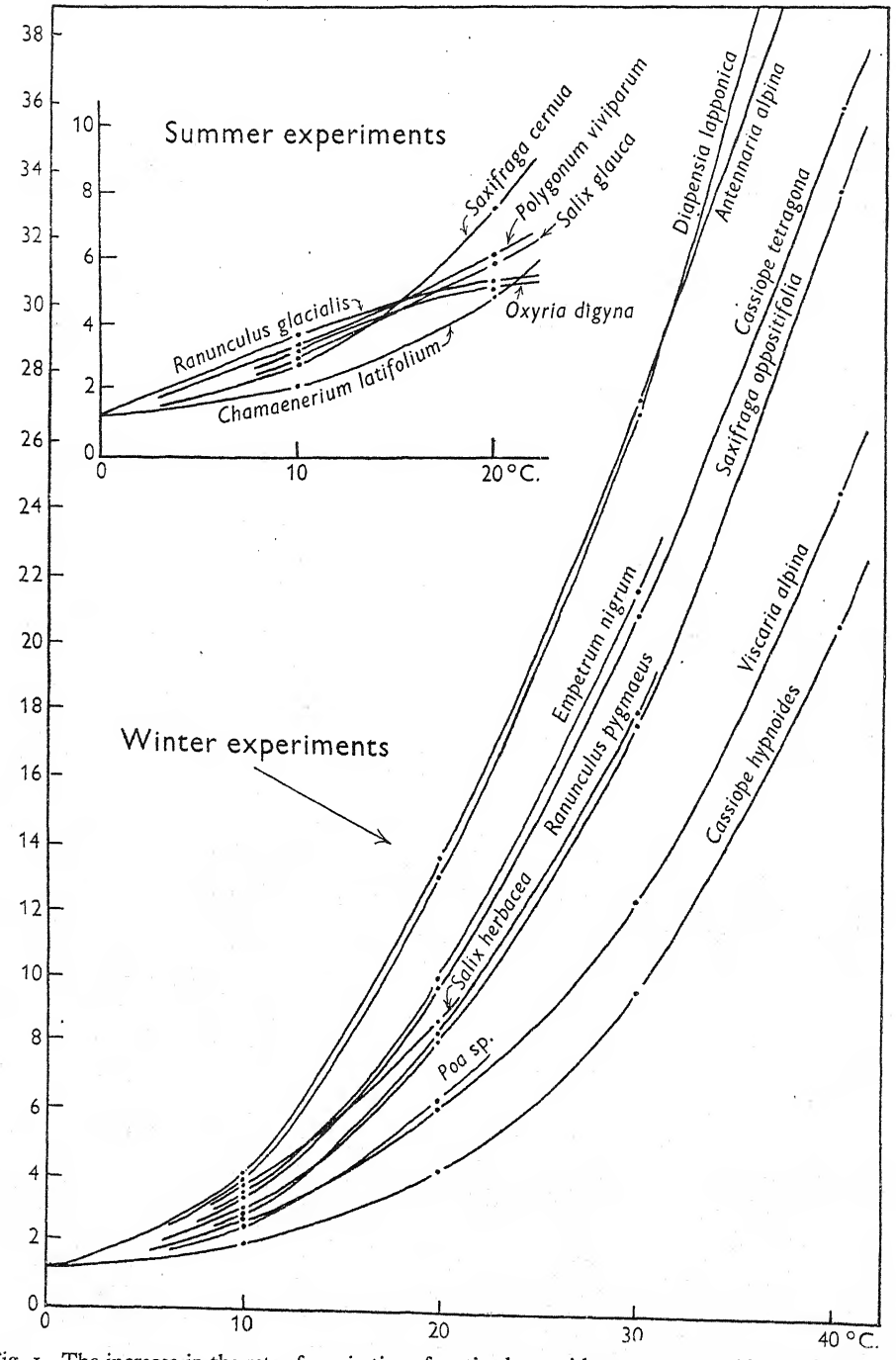

Fig. 1. The increase in the rate of respiration of arctic plants with temperature. Abscissa: temperature; Ordinate: rate of respiration relative to a value of unity at 0° C.

It is worth pointing out that these curves, with the exception of that of *Cassiope hypnoides*, do not fit any of the formulae proposed relating the rate of a chemical process to temperature, such as the Arhenius equation or the Van't Hoff law, because in these results the Q_{10} falls with increase of temperature (*Cassiope hypnoides* shows a constant Q_{10} from 0–40° C.). Bělehrădek (1930) has proposed an empirical equation relating the rate of a process to temperature which in effect contains three arbitrary constants. The present data can be fitted to such a type of equation with a fair degree of success, but with the inevitable narrow range of temperature and the use of three constants little seems to be gained by such an analysis, which serves neither to elucidate nor to systematize the facts.

It may be seen from Fig. 1 that the curves constructed from the summer material are on the average lower than those for the winter material. The Q_{10} values have been averaged and, including Müller's summer ones, are as follows:

	0–10° C.	10–20° C.	20–30° C.	30–40° C.
Winter	3·1	2·7	2·1	1·9
Summer	2·9	2·3		

These figures show that, at any rate in this collection of data, the Q_{10} of the summer material falls well below that of the winter in the 10–20° C. range, and that it differs but little in the lower range of 0–10° C. Table 2 is a collection of data for such temperate plants, avoiding storage organs, as could be found, and it is seen that the averages fall close to those for the summer plants in Greenland.

Table 2. *Temperature coefficients of respiration rate of temperate plants*

Observer	Experimental material	0–10 ° C.	10–20 ° C.	20–30 ° C.	30–40 ° C.
Briggs *et al.* (1920)	*Zea mays* plants	2·8	2·2	—	—
Brown & Escombe (1905)	*Helianthus annuus* leaves	—	—	2·4	1·7
Bushnell (1925)	*Solanum tuberosum* leaves	—	—	1·8	—
Clausen (1890)	*Triticum sativum* seedlings	2·9	—	—	1·1
Fernandes (1923)	*Pisum sativum* roots	2·9	2·2	2·2	1·1
Kidd *et al.* (1921)	*Helianthus annuus* leaves	3·3	2·2	—	—
Lundegårdh (1924)	*Solanum tuberosum* leaves	3·3	1·9	2·1	2·1
Lundegårdh (1927)	*Beta vulgaris* leaves	—	1·8	2·3	1·8
Matthaei (1905)	*Prunus laurocerasus* leaves	—	2·2	2·3	—
Mayer (1876)	Etiolated *Triticum sativum* seedlings	3·5	—	1·5	—
Newton & Anderson (1931)	*Triticum sativum* leaves	2·8	—	—	—
Wager, Table 1	*Empetrum nigrum* shoots	3·5	2·7	2·3	2·0
Wager, Table 1	*Saxifraga oppositifolia* shoots	3·9	2·5	2·1	1·8
	Average	3·2	2·2	2·1	1·75

So far the discussion has been concerned only with the rate of increase of respiration with temperature, and it is this that is lower for the summer plants. The actual rate of respiration, as may be seen from Table 1 and Fig. 3, tends to be higher for the summer plants, and this will now be considered.

The lower rate of respiration of the winter material is probably to be correlated with the facts that it includes stems and that the leaves were of necessity old. In contrast to this the summer material was all medium-aged leaves with no stalks and very little petiole. These differences in type and age of the summer and winter material should be sufficient to give the differences in the rates of respiration, but they should not necessarily affect the values of the Q_{10}, and the explanation of the larger winter values of this is believed to be on the following lines.

The osmotic pressure of the Greenland plants tested rose markedly in the autumn and fell again in the spring (Wager & Wager, 1938*a*) and it would seem to be a reasonable deduction that the soluble carbohydrates rose and fell also (cf. work in temperate regions by, *inter alia*, Dixon & Atkins (1915), Lewis & Tuttle (1920), Goverov (1925), Steiner (1933) and Schaffnit & Wilhelm (1933)). It was further shown that when *Vaccinium uligonosum* was brought inside during the winter its osmotic pressure fell, and so probably its soluble carbohydrates fell also, to its summer values in about 15 days.

Thus the higher winter level of carbohydrate concentration was probably largely maintained for the whole of a normal respiration run. If this be true, two effects might be expected to follow:

1. The higher sugar concentration brought about by the low temperature should lead to a higher rate of respiration per unit weight of the winter material.

2. There should be a relatively greater increase of respiration at higher temperatures in winter plants compared with summer plants, because the enzyme system could be kept more fully saturated due to the larger substrate concentration, and so a larger Q_{10} would be expected for the winter plants especially over the higher temperature ranges.

In the experiments described here the first effect is not shown for the reasons already given, but it is believed that an effect due to a similar cause shows when comparison with a group of temperate plants is made, due to the lower summer temperatures to which arctic plants are subjected[1] (see p. 9).

The second effect, namely that of a larger Q_{10} in the winter plants, is clearly shown (see average Q_{10} values quoted on p. 7), and supporting evidence may be obtained from an examination of the change in value of the Q_{10} for the same temperature interval during the course of a single respiration run. In general, the rate of respiration falls with time during a run, as starvation effects gradually develop and a fall in the Q_{10} with time would also be expected if point 2 above is correct. From the data collected, sixteen pairs of comparisons for the same material over the same range of temperature at different periods of the starvation curve can be made. Of these sixteen comparisons, twelve show a decrease with time and four an increase. Two of those showing an increase are for *Cassiopé hypnoides*, the only material in these experiments which showed a continuous rise in the 20° C. respiration rate:

[1] After the above ideas about the concentration of soluble sugars and respiration rates were worked out Mr R. Scott Russell told me that he had found a high concentration of soluble sugars in the plants in Jan Mayen Island, in the summer of 1938, and thus he has supplied direct evidence for the high sugar concentrations that were inferred from the osmotic pressures of the arctic plants and the behaviour of plants during frost hardening in temperate regions.

one of the remaining values depends on a difference of 0·04 and 0·038 c.c. of acid per hr. for the 0° C. rate and so is barely significant. Thus, with only one significant exception, all the readings agree in showing a drift of Q_{10} in the same direction as the drift in rate of respiration with time, and so are in agreement with the suggestion that the value of the Q_{10} depends to some extent on the supply of substrate for the respiratory mechanism.

In agreement with these ideas Newton & Anderson (1931) find that in different races of wheat, those which resist frost best develop the highest concentration of soluble carbohydrates and have the highest Q_{10} of respiration over the ranges −7–0° C. and 0–+7° C. (the only ranges studied by them).

Many of the rates of respiration of the Greenland plants seemed rather high, so a comparison with a representative group of temperate plants was undertaken. This question has been considered by Stocker (1935) using leaf area as the basis for expressing the respiration rates. He gives a long table of respiration rates and concludes that the rate of respiration of tropical trees is lower than that of temperate trees or than that of *Salix glauca* and *Betula nana*, the only arctic trees studied. If the values of respiration rates quoted by him are averaged, including the four new arctic values, the following figures are obtained for the rates of respiration at 20° C.:

Arctic: 2·3 mg. per sq. dec. per hr. (average of 6)
Temperate: 1·25 mg. per sq. dec. per hr. (average of 42)
Tropical: 0·5 mg. per sq. dec. per hr. (average of 5).

Leaf-area is not a good unit to use when comparing respiration rates, and so an attempt has been made to make the comparison on a fresh-weight basis. All the data that the writer has been able to find are plotted in Fig. 2 and the corresponding data for arctic plants in Fig. 3. The values for the rates of respiration of temperate plants were obtained from those papers marked with an asterisk in the list of references at the end of this paper.

There are obvious difficulties in drawing conclusions from this data. One of the most important is the well-known fact that the rate of respiration of an organ decreases with its age (Kidd *et al.* (1921), Hover & Gustafson (1926)). This is not a uniform decrease in rate with time, and in some cases, at any rate, there is a period of slow change corresponding to maturity of the organ, so random adult leaves should give a more just estimate of respiration rate than might at first sight be supposed.

Many of the arctic values are for winter plants and so the respiration rate should be low, as the plants were relatively senescent, and further, six out of the seventeen of the arctic values are from material which included woody stems and so the rates obtained should be low (as already pointed out, winter respiration rates are lower than summer ones). Another point is that the respiration rates for temperatures above 20° C. are lower than their true rates, as they were determined late in the course of the respiration runs. As a result of these factors the differences between the arctic and temperate rates of respiration should be appearing at a minimum in these graphs.

Bearing these points in mind the writer would draw the provisional conclusion that the graphs of arctic and temperate respiration rates (Figs. 2 and 3) and the

averaged values calculated from Stocker's tables show that the leaves of arctic plants have on the average a higher rate of respiration than leaves of temperate plants at the same temperature. It is extremely probable that this generalization could be extended to cover the temperate and tropical groups of plants, but at present the data available for tropical plants is too scanty.

It is possible that the rate of physiological ageing is not the same at the lower temperature prevailing in the arctic, and that this difference in average respiration

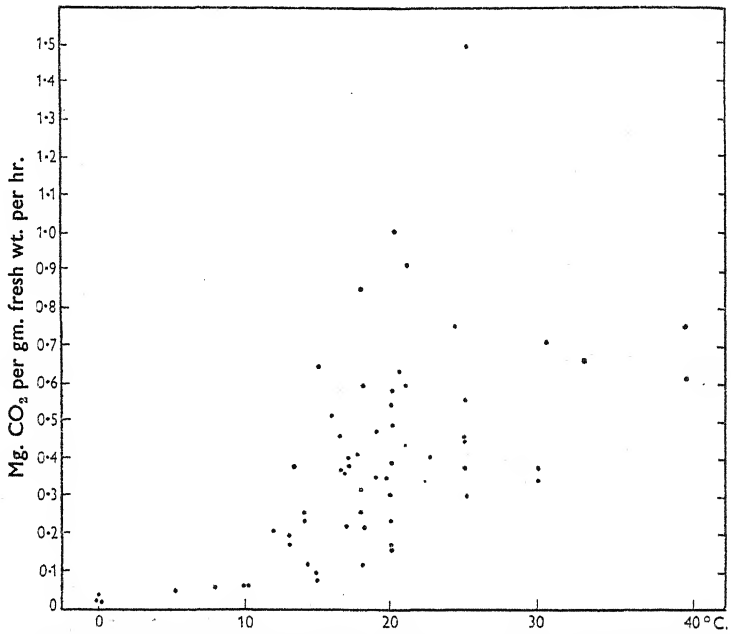

Fig. 2. The collected values of the respiration rates of leaves of temperate plants plotted against the temperatures at which they were determined. (Data from papers marked with an asterisk in the references at end of paper.)

rate is to be associated with this factor rather than with a difference in the basal rate of respiration, but even so the difference would remain as a point of considerable ecological importance.

The writer is inclined to associate these differences in the average respiration rates with the probable differences in the carbohydrate concentrations which have been postulated for plants in colder regions rather than to any alteration of the respiratory enzymes.

Results and discussion of the carbon assimilation experiments

The intention of studying the assimilation-temperature-light curves of a representative group of arctic plants could not be carried out due to wilting of the leaves of many plant species in the assimilation chamber and to the fact that many arctic plants have unsuitable types of leaf for comparative studies of this type. Fair success was attained with *Oxyria Digyna*, *Ranunculus glacialis* and *Saxifraga cernua*.

The results of the successful runs on these plants are plotted in Figs. 4, 5 and 6 in terms of mg. of carbon dioxide per hr. per 50 sq. cm. of leaf area (100 sq. cm. of leaf surface). It is fairly certain that none of the values plotted are for wilted material. The rather low values for the maximum rate of assimilation for the 20° C. experiment on *Saxifraga cernua* are probably due to the late date of the experiment, 19 July, by which time the plants from which the leaves were gathered were getting

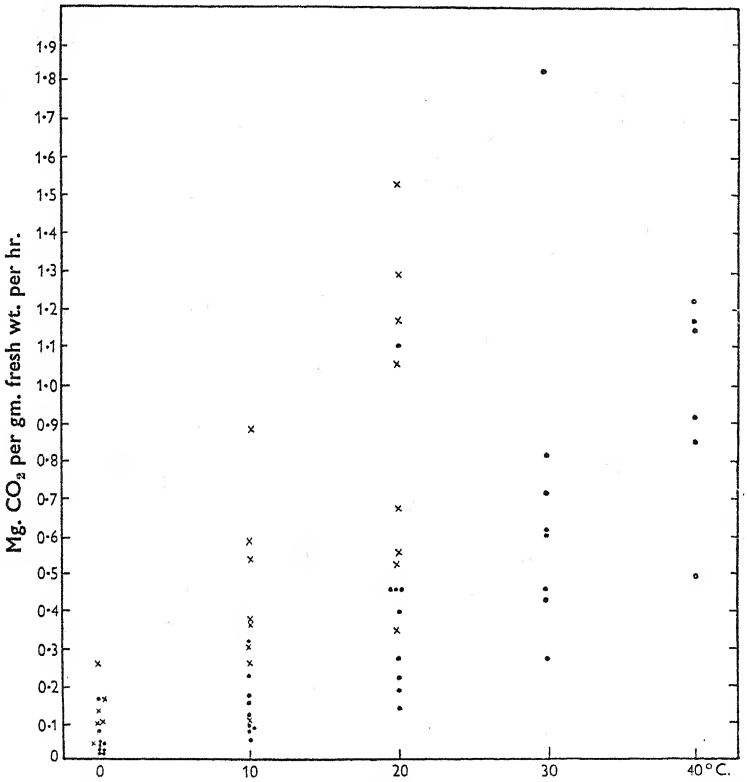

Fig. 3. The collected values of respiration rates of leaves of arctic plants plotted against the temperatures at which they were determined. Summer determinations × ; winter determinations •.

rather senescent. A table giving the dry weight of one gram of fresh weight of the leaves is given (Table 3). The ratios of fresh and dry weight to leaf area were sufficiently constant to make the individual quotation of values for each experiment unnecessary.

Table 3

Species	Dry wt. of 1 gm. fresh wt.	Leaf area of 1 gm. fresh wt. in sq. cm.
Oxyria digyna	0·10	17·9
Saxifraga cernua	0·09	21·8
Ranunculus glacialis	0·14	18·9

Description of the curves in detail is unnecessary, but a few general points may be made. *Oxyria digyna* appears to be a fairly typical "sun" plant with a high rate of assimilation and of respiration, whilst *Ranunculus glacialis* and *Saxifraga cernua* are intermediate in type with lower rates of assimilation and high rates of respiration. The curve given by Müller for *Chamaenerium latifolium* is very similar to that given here for *Ranunculus glacialis*, but his curve for *Salix glauca* attains a maximum assimilation rate of only 3 mg. per 50 sq. cm. per hr., yet it has a high rate of

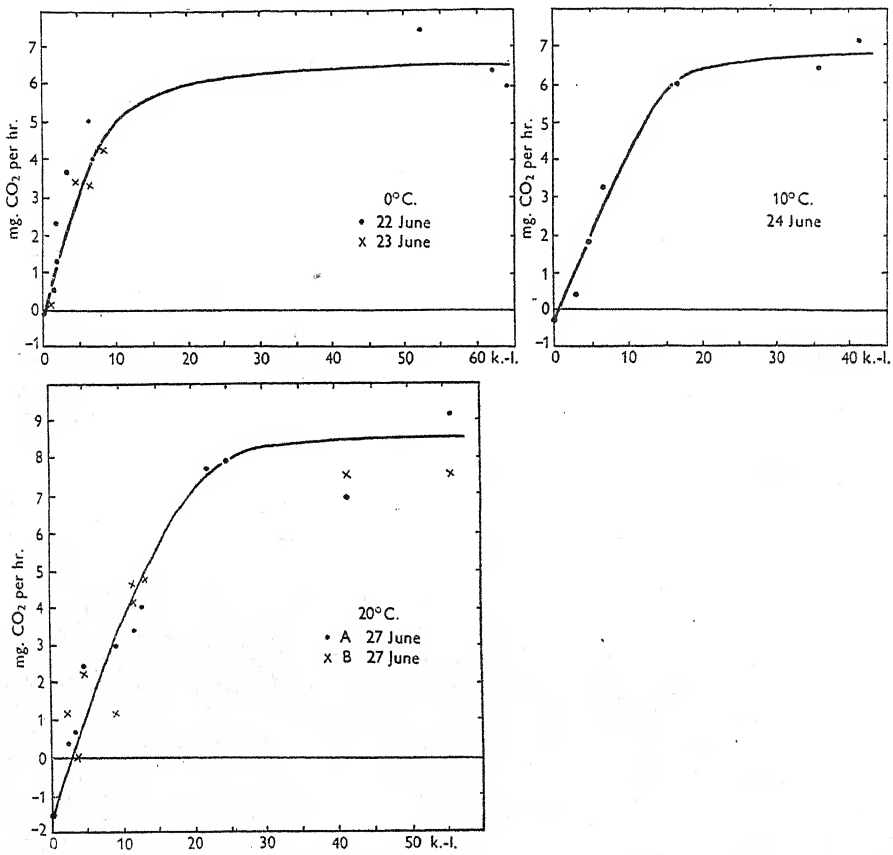

Fig. 4. Rate of apparent assimilation of *Oxyria digyna*, expressed as mg. of CO_2 per 50 sq. cm. of leaf area per hr., plotted against the light intensity in kilo-lux, for three temperatures.

respiration. In consequence of these high rates of respiration the compensation points of all these plants at 20° C. are fairly high, even though the maximum assimilation rate is low. On the other hand, at the normal low temperatures of their environment, and especially with night temperatures when the light intensity is low, the compensation points are also low, as may be seen from the curves for 0 and 10° C.

The maximum assimilation rate at 20° C. of the arctic plants so far studied falls between 3 mg. of carbon dioxide per hr. per 50 sq. cm. of leaf area (*Salix glauca*,

Müller, 1928) and 9 mg. (*Oxyria digyna*) and may be compared with 2 mg. (*Oxalis acetosella*, Lundegårdh, 1921) and 10 mg. (*Solanum tuberosum*, Lundegårdh, 1931) for temperate plants. The two ranges are about the same, so that no special adaptation is shown by arctic plants in this direction.

The Q_{10} of apparent assimilation of arctic plants may be compared with such values as could be found in the literature for temperate plants (Table 4). Some of these values seem to be unsatisfactory, as it is difficult to see why the Q_{10} should be so large under conditions of high light intensity and low carbon-dioxide concentra-

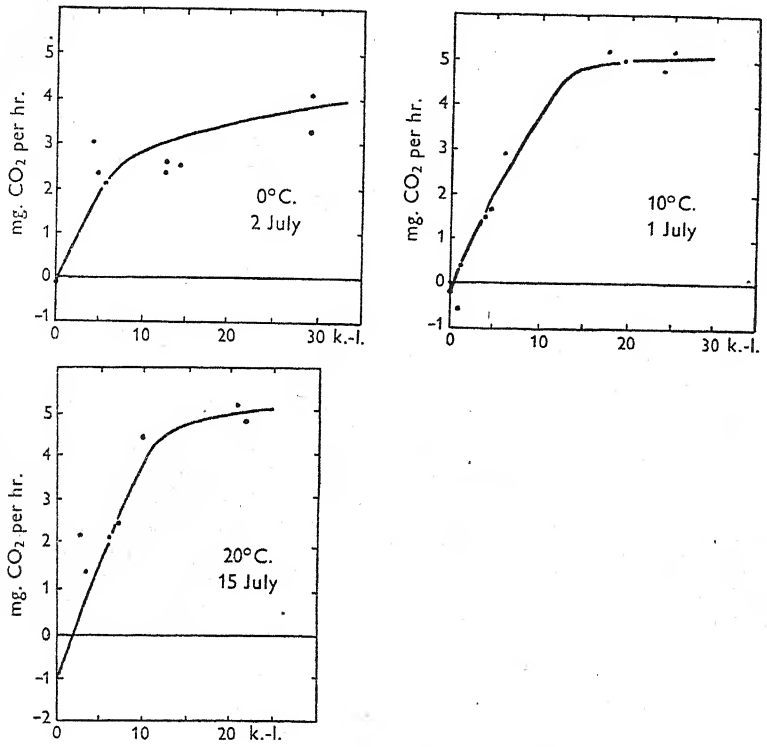

Fig. 5. Rate of apparent assimilation of *Saxifraga cernua*, expressed as mg. of CO_2 per 50 sq. cm. of leaf area, per hr., plotted against light intensity as kilo-lux for three temperatures.

tion. The possibility that these values may be in part an expression of stomatal movement or some other factor not directly part of the assimilatory mechanism must be considered. Nevertheless, it seems justifiable to suggest as a preliminary conclusion that the Q_{10} of apparent assimilation with high light intensity and low carbon-dioxide concentration is lower for arctic than for temperate plants. The number of figures in the table is small, but there is little overlapping of values, and even if the two most improbable of the temperate values, 4·8 and 5·1, are excluded from the comparison the conclusion is unaffected. The fact found by Harder (1925) that, in water plants cultivated at different temperatures, the high-temperature plants had the largest Q_{10} of assimilation, is in agreement with the conclusion

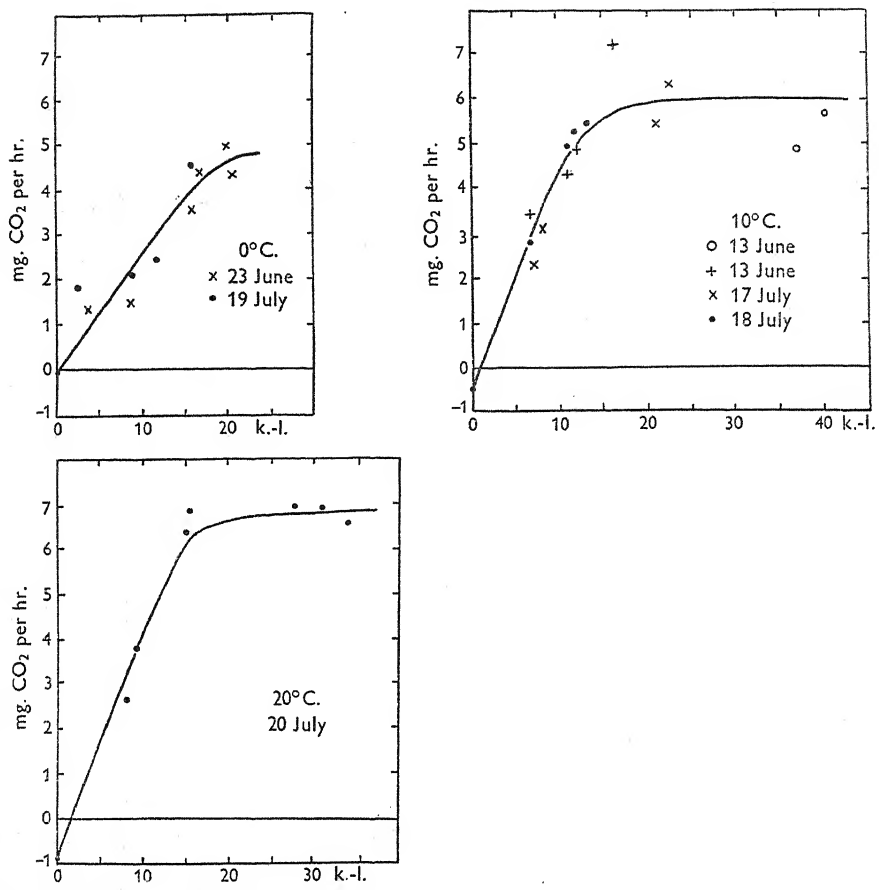

Fig. 6. Rate of apparent assimilation of *Ranunculus glacialis*, expressed as mg. of CO_2 per 50 sq. cm. of leaf area per hr., plotted against light intensity in kilo-lux for three temperatures.

Table 4. *Temperature coefficients of carbon assimilation of arctic and temperate plants*

	Q_{0-10}	Q_{10-20}	Observer		Q_{0-10}	Q_{10-20}	Observer
Oxyria digyna	1·1	1·3		*Solanum tuberosum*	4·8	2·2	Lundegårdh (1924)
Ranunculus glacialis	1·1	1·1		*Solanum lycopersicum*	1·8	1·4	Lundegårdh (1924)
Saxifraga cernua	1·4	1·0		*Beta vulgaris*	2·0	1·4	Lundegårdh (1927)
Salix glauca	—	1·3	Müller (1928)	*Anemone nemorosa*	1·9	0·9	Lundegårdh (1927)
Chamaenerium latifolium	—	1·2	Müller (1928)	*Vicia faba*	5·1	1·6	Walther (1927)
				Phaseolus vulgaris	1·4	2·0	Yoshii (1928)
Average	1·2	1·2		Average	2·9	1·7	

reached above and makes the possibility that the effect is part of the physical system rather than the assimilatory mechanism rather less likely. On the other hand, Harder's result indicates that this low Q_{10} of assimilation found in arctic plants may be a direct modification that any plant growing in the arctic would undergo rather than a peculiarity of arctic plants.

These assimilation results appear to have some bearing on the problem of "sun" and "shade" leaves. Considered from a physiological point of view these two types of leaf seem to be ill-defined, and the concept seems to have been based on a few typical examples, such as *Oxalis acetosella* and *Nasturtium* or *Beta*: in such cases the two types of leaf show clear-cut differences. The "sun" type of leaf has a high rate of maximal assimilation, a high rate of respiration and in consequence a relatively high light intensity at the compensation point: the shade leaf has the reverse in each instance, but exceptions occur in temperate regions (see for example the ferns investigated by Johansson (1923, 1926). For the arctic plants investigated the relationship between respiration and assimilation does not hold good either, as *Salix glauca* has one of the largest rates of respiration recorded for adult leaves and yet its rate of assimilation is that of a "shade" plant, and the lowest so far recorded for the arctic. This is the most striking example, but the rates of assimilation and respiration of the other plants do not fall in the same sequence.

All arctic plants grow in the same light climate and should be regarded as "sun" plants since there is no shade in the arctic, yet even so they show the same range in maximum assimilation rate as plants described as "sun" and "shade" types in temperate regions. Thus it seems reasonable to suggest that light is not perhaps the primary conditioning factor for the production of "sun" and "shade" plants and that the group of plants found growing in any locality will show a large range of variation between these two extreme types. It may be that plants which have a low rate of respiration and of maximum assimilation are more tolerant of shade and so grow there.

Discussion of the results in relation to the distribution of arctic and temperate plants

It is interesting to consider whether the results reached above give any indication of the factors that lead to the distribution of arctic and temperate plants. The relevant broad generalizations arrived at are:

1. The rate of assimilation at 20° C. is about the same for arctic and temperate plants.

2. The rate of assimilation is less diminished for arctic than for temperate plants by temperatures lower than 20° C., i.e. the Q_{10} of apparent assimilation is smaller.

3. The respiration rate of arctic plants is greater at all temperatures than of temperate plants, but the overlap in respiration rates of the two groups is considerable.

The differences considered in these generalizations are not large, nor are they

clear cut because of the scatter and overlapping of values, and so would not appear to be sufficient in themselves as an explanation of the factors causing the distribution of arctic and temperate plants. At the same time they do indicate that an arctic plant would have a greater suitability for the arctic climate than would a temperate plant. Two provisos must be added to this last statement: it takes no account of the possibility of the direct modification of the metabolism of the plant in the directions indicated by temperature (cf. Harder's results), and, owing to the overlap in the values from the arctic and temperate plants, some individuals will be found that appear to be in direct contradiction to these statements. Thus, by themselves the rates of respiration and assimilation do not appear to be adequate to explain the plant distribution, nor can the intensified action of these due to interaction with factors such as ecological competition be the explanation, as some plants have a southern limit in the arctic in places where no competition, other than very slight root competition, is present.

Müller (1928) has suggested that the long warm night of temperate regions is the factor limiting the southern distribution of arctic plants. This is improbable, as some arctic plants can be transplanted to temperate regions and there continue active and healthy growth, and the long warm night is counterbalanced by the very much longer growing season in temperate regions.

The short growing season of the arctic plants will greatly restrict their growth rate in the arctic (for a discussion of this see Wager & Wager, 1938*b*), but not their capacity to grow in temperate regions, and so cannot be used as an explanation of the southern limit of growth. On the other hand, it may be a primary factor in restricting the northward spread of many species. The southern limit of distribution appears to be a more difficult problem at present than the northern one.

Among other factors that might influence the growth of plants in the arctic, one appears to the writer to be of great importance, namely the capacity of the plants to come into leaf and flower very rapidly in the spring at the rather low temperatures then prevailing. This is very largely expansion of preformed organs, as autumn buds are well developed, but all phases of growth probably occur. As a result of this rapid growth the plants are enabled to make full use of the short growing season. Both the high rate of respiration, previously suggested to occur in arctic plants, by allowing a high rate of synthetic metabolism, and the high concentration of soluble carbohydrate postulated from the osmotic pressure determinations would favour a high rate of growth. Thus, indications of the significance of these facts for growth in the arctic are found.

It might finally be suggested that one of the reasons why certain plants can survive in the arctic is that they have the capacity for increasing their general metabolic rate and their soluble carbohydrate concentrations to a greater extent than other plants.

Further speculations on the basis of the rather meagre data at present available are not profitable, and those that have been made are in the hope of suggesting possible lines of physiological investigation in the arctic.

Summary

1. The rate of respiration of ten plants growing under arctic conditions at Kangerdlugssuag, East Greenland, was determined during the winter at various temperatures by estimation of the carbon dioxide given out by the plant. The respiration rate of five plants was determined during the summer.

2. From the results it is concluded:

(*a*) The Q_{10} of the rate of respiration is larger during the winter than during the summer.

(*b*) The Q_{10} of the rate of respiration of the arctic winter plants is larger than that of temperate plants.

(*c*) The rate of respiration per gram fresh weight of arctic plants is larger than that of temperate plants.

3. The rate of carbon assimilation of three plants was measured at 0, 10 and 20° C. at various light intensities. The results show that:

(*a*) With normal carbon-dioxide concentration of the air and high light intensity the range in the rate of assimilation in arctic and temperate regions is about the same.

(*b*) The Q_{10} of the rate of apparent assimilation with high light intensity of arctic plants is less than that of temperate plants.

4. The bearing of these results on the question of "sun" and "shade" leaves and on the factors controlling plant growth in the arctic are considered.

Addendum

Since this paper went to press there has been published a paper by Mr R. Scott Russell (Physiological and Ecological Studies on an Arctic Vegetation. III. *J. Ecol.* **28**, 289, 1940) on the physiology of plants in Jan Mayen Island. Mr Scott Russell shows that the concentration of soluble carbohydrates in the three plants tested was high compared with those found in temperate plants and that the 'nett assimilation' rate was about the same as for temperate plants. These results are in agreement with the conclusions put forward in the present paper.

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THE BIOLOGICAL DECOMPOSITION OF CELLULOSE

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I. Introduction

The constitution of cellulose has been established as the result of the observations of many workers. Detailed accounts of the structure of the molecule, the larger units into which the molecules are aggregated and the evidence on which these are based can be found in Haworth (1934), Freudenberg (1933) and Norman (1937). The structure of cellulose in relation to the cell walls of which it forms a part has been recently considered by Preston (1939) and Frey-Wyssling (1939).

Briefly, the molecule of pure cellulose is composed of β-glucose units linked through the 1·4 positions to form long chains. The recorded molecular weights, obtained by the use of different techniques, cover a wide range. Expressed in terms of the number of glucose units in each molecule, the range extends from the minimum value of 100–200 glucose units (Haworth & Machemer, 1932) to 700–800 units (Staudinger, 1933), while a third method (Neale, 1936) gives a chain length of 1200 units. Freudenberg (1932) considers that the cellulose molecules are grouped together into micelles by secondary valencies, the micelles being 500–1000 A. in length and 60 A. thick, and aggregated into homogeneous groups—micelle series—of the order of 600 A. in thickness. These groups compose the lamellae of the cell walls.

In the natural cellulose of plant tissue the pure cellulose is closely associated with certain other complex polysaccharides, which Hawley & Norman (1932) have described as cellulosans. Xylan is the most widely distributed of this group and is common to both angiosperms and gymnosperms, being associated in the latter with a mannan. The cellulosan content of natural cellulose varies from species to species, it is greater in hard than in soft woods and in certain cases is 30% of the total cellulose.

Cellulose can serve as a source of energy to many organisms, including certain Invertebrate animals, fungi, bacteria and the germinating seeds of the angiosperms.

Before it can be utilized by any of these organisms it must be broken down to water-soluble molecules, which can diffuse into the cells. This degradation previous to oxidation by the cell respiratory system, is enzymic, mainly hydrolytic, and is known to be the work of more than one enzyme. It is the purpose of this review to consider the nature of the enzymic systems responsible for this preliminary attack and their specific characteristics. The later stages of the breakdown, probably wholly oxidative, will not be considered as they are probably the same in any one organism, whatever the nature of the carbohydrate initially attacked, and not specific to cellulose.

The decomposition of such complex cellulose units as those with which the lamellae of the walls of cells and fibres are built must proceed in at least two stages. First, the bonds which unite the molecules of cellulose into macro-units must be broken, and secondly, the linkages which unite the glucose units into the cellulose chain must be dissolved. In biological studies these two stages cannot be examined separately, as they proceed simultaneously in different parts of the same tissue or even in the same macro-unit or micelle series. Whatever biological agent is used for the decomposition of cellulose, it is universally recorded that the greater the degree of dispersion of the cellulose the higher is the rate of attack. The degree of dispersion may be regarded as the extent to which the macro-units of cellulose have been broken down to the free molecules. Sellière, in a series of papers (1905–10), examined the hydrolysis of cellulose materials by enzyme action, the product being glucose. He found that cellulose was attacked more rapidly if it were first treated with 25 % caustic soda or with zinc chloride or precipitated from Schweitzer's reagent. He concluded that the physical condition of the cellulose was an important factor in determining the rate of hydrolysis under enzyme action. In much of the work on this subject the cellulose used as a substrate has undergone some form of pre-treatment which increases the degree of dispersion and which increases the rate at which the substrate is broken down to easily identified end products. The physical structure of cellulose aggregates as they occur in plant tissue may therefore be regarded as offering a measure of protection against destructive agents. This is true not only in the biological sphere but also in that of purely chemical activity. The form in which cellulose is used for the preparation of the methyl derivative is, for example, a factor of considerable importance in determining the amount of the derivative formed under constant experimental conditions.

Cellulose-decomposing enzymes from three sources will be considered, the germinating seeds of angiosperms (whether cellulose is a storage product or only a constituent of the cell wall), bacteria and fungi.

II. The cellulase activity of germinating seeds

Sachs (1862), as the result of observations on changes in the reserve cellulose during the germination of the seeds of the date, *Phoenix dactylifera*, supplied evidence of the presence in this seed of a cellulose hydrolysing enzyme. The reserve cellulose in this seed takes the form of heavily thickened cell walls in the endosperm,

and during germination they show signs of dissolution in the neighbourhood of the epithelium which covers the surface of the developing cotyledons. As the cotyledons push into the endosperm, so the cell walls are dissolved. Green (1887) recorded the isolation of a cellulose-dissolving ferment from the germinating date seed. Brown & Morris (1890) were unable to confirm this observation. These two workers were the first to examine the cellulose hydrolysing enzymes in germinating barley or malt. They investigated the changes which occur in germinating seeds, barley seed being dealt with in considerable detail. Their results were obtained chiefly by the use of microscope technique. They showed that in the germinating barley seed the cell walls in the endosperm were dissolved in advance of the enzymic attack on their contents. The cellulose-fermenting enzyme was extracted from dried barley malt in aqueous solution and was capable of decomposing the endosperm cell walls of all members of the Gramineae including the abnormally thick ones of *Bromus mollis*. It had, however, no effect upon the thick endosperm cell walls of *Phoenix dactylifera*, *Asparagus officinalis*, *Coffea arabica* and *Allium cepa*; in all these species the thick walls are a reserve food substance. The enzyme extract was capable of hydrolysing the walls of the cells of potato tubers leaving only the intact middle lamellae which are composed of a pectic substance and not cellulose. This activity was also observed using slices of artichoke, carrot, turnip and to a lesser extent beet. The source of the enzyme in the seed appears to be the columnar epithelium of the scutellum. Excised barley embryos attacked slices of potato tuber and the fibres of filter paper with which they were in contact. These detailed observations form the basis on which later examinations of cellulase activity are based.

Pringsheim & Siefert (1923) hydrolysed lichenin isolated from *Cetraria islandica* with an enzyme prepared from barley malt. The end product was glucose, identified by the melting point of its osazone and its rotation in solution.

Pringsheim & Bauer (1928) prepared aqueous extracts of malt, and purified and separated the enzymes present by adsorption on and elution from kaolin and aluminium β-hydroxide. They found that the purity of the substrate was of importance in the study of the kinetics of the fission. With pure lichenin the monomolecular law was followed up to 60% hydrolysis. The cellobiase present followed the same law. Both reactions are therefore brought into line with the reaction kinetics of most disaccharides. They brought forward evidence of the importance of the degree of dispersion of the substrate as a factor determining the rate and extent of the hydrolytic enzyme attack. They used four dispersions of cellulose, two prepared by using lithium chloride (Li 1 and Li 2), one by using calcium thiocyanate (R) and one by using 62–63% sulphuric acid as the reagent in the pretreatment. The last method followed the technique of Schwalbe and Schultz giving a product known as "Guignet-cellulose" (G). Table 1 gives their results.

Extracts prepared from malts of barley, rye and wheat all hydrolyse lichenin to a considerable extent, 40–76%, and all contain a cellobiase which hydrolyses cellobiose to the extent of 79–100%. Karrer & Staub (1924) isolated lichenase from germinating barley, the amount present in the seed being constant between the 2nd and the 11th day of germination. It was also found in the germinating seeds of

wheat, spinach and maize. In the last species during the first days of germination the amount was evenly divided between endosperm and embryo, subsequently the embryo contained the larger amount.

Table 1. *Fission in terms of % yield of glucose.*

Length of hydrolytic action	Li 1	Li 2	R	G
16 hr.	8·6	10·4	21·4	3·8
24 hr.	12·4	14·4	25·7	6·0

III. The activity of bacteria and fungi in the decomposition of cellulose

The study of the decomposition of cellulose by bacteria and fungi will be separated into two parts, the first dealing with the cellulose decomposing activity of isolated enzyme preparations and the second with the destructive activities of the organisms as the result of their normal physiological function. The product of the action of isolated enzymes is usually glucose in almost quantitative yield, the utilization of this substance being the work of the whole organism. When the activities of the whole organisms are examined, glucose is rarely found as an intermediate product. It is obvious that intermediate substances will accumulate in the medium only in so far as the later stages in the breakdown of cellulose proceed at a slower rate than the earlier. Such incomplete linkages between the stages of cellulose breakdown by the "brown rot" fungi leads to a temporary accumulation of an intermediate, the analysis of which has given valuable information concerning the mechanism by which these fungi attack cellulose.

(1) *Isolated enzyme preparations*

In 1888 Marshall Ward reported from microscopic observations that the hyphae of *Botrytis* sp. appeared to secrete an enzyme capable of hydrolysing the cell walls of the host immediately in front of the tips of the hyphae. This was the first record of the activities of an exoenzyme of the cellulase group. Similar observations have been made on other parasites and hosts. While it appeared obvious that plant tissues and timber could only be attacked by fungi and bacteria capable of secreting a cellulase, a number of results were published which showed that in certain bacteria the cellulase was of the endoenzyme type, inactive in the absence of the whole cell. Pringsheim (1912*a*) found that the decomposition of cellulose by certain thermophilic bacteria occurred in two stages, cellulose to cellobiase and cellobiase to glucose. The enzymes concerned were of the endoenzyme type and cell-free extracts had no action on cellulose. It was only possible to employ the activity of these enzymes apart from the normal respiratory action of the bacteria by destroying the vital function of the bacteria by the use of antiseptics. Since this first report of endocellulase and cellobiase a number of reports of both types of enzyme in bacteria have appeared. A large number of fungi have been examined in respect of

their enzyme content, but in few cases has any work been done on the kinetics of the reactions or the exact relationship between substrate and enzyme under varying conditions. The wood-rotting Polypores have been examined by a number of workers, Schmitz (1920, 1921, 1925), Lutz (1930, 1931), Nutman (1929), and most recently Bose & Sarkar (1937). In all cases our knowledge of the distribution of enzymes in fungi has been increased but little or no advance has resulted in our knowledge of the factors controlling the activity of any individual enzymes. Our knowledge in this direction has been increased mainly as the result of work on enzymes extracted from certain species of moulds. Grassmann & Rubenbauer (1931) stated that a dialysed and concentrated enzyme extract from *Aspergillus oryzae* hydrolysed both filter and parchment papers at a slow rate. Grassmann, Stadler & Bender (1933) found that a crude extract of this fungus hydrolysed hydrocellulose, cellulose dextrins, lichenin, mannan, inulin and xylan. This preparation hydrolysed a cellulose prepared from beech wood at a slow rate and a hydrocellulose prepared from this source and a hydrocellulose prepared from cotton about 25 times as fast. A dialysed preparation free from cellobiase hydrolysed hydrocellulose, cellulose dextrins and lichenin with optimum speed at *p*H 4·5. This value is quite distinct from the optimum value 5·2 recorded for the lichenase activity of Karrer's snail and Pringsheim and Bauer's malt enzymes. They stress the importance of taking into account the differences between the physical conditions of the substrates when comparing cellulase and lichenase activities. Grassmann, Zechmeister, Toth & Stadler (1933) separated a cellulase and cellobiase from a crude enzyme extract of the same species, *A. oryzae*. On examination the cellobiase proved to be an oligo-saccharase splitting not only cellobiose but cellotriose, cellotetraose, and cellohexaose. The cellohexaose, with a molecular weight of 990, was the largest molecule attacked. The cellulase was a polysaccharase hydrolysing not only cellulose but also cellulose dextrins with a lower limit of molecular weight of about 1000. Table 2 shows the sharp cleavage between the two zones of activity.

Table 2

Substrate	Fission after 24 hr. in terms of c.c. $N/50$ iodine	
	Polysaccharase	Oligosaccharase
Hydrocellulose	0·55	0·10
Cellodextrin	2·57	0·20
Cellohexaose	2·98	5·35
Cellotetraose	0·10	3·50
Cellotriose	0·50	2·70
Cellobiose	0·00	2·90
Xylan	1·60	0·40

This is a most important observation and its significance in association with results obtained with cellulases from other sources will be considered later.

Freudenberg & Ploetz (1939) examined an enzyme extract from a technical preparation "Luicym" prepared from *Aspergillus oryzae*. They separated a lichenase, cellulase and cellobiase from the crude extract. The individuality of the first two

was clearly established by the wide separation of their *p*H optima, 5·9 and 4·7 respectively. The wide difference between the value of 5·9 which they report and the 4·5 recorded by Grassmann, Stadler & Bender (1933) for the *p*H optimum of lichenase remains to be explained. In view of the different techniques used, the possibility does arise that more than one enzyme was necessary for the hydrolysis of the substrate. Such enzyme systems might well show different *p*H optima as the enzyme limiting the rate of hydrolysis in the two preparations might not be the same in both.

Timber-rotting fungi are commonly divided into two groups, "brown" rots which attack cellulose but not lignin and "white" rots which attack both lignin and cellulose. The activity of the former group has been regarded as mainly hydrolytic, that of the latter both hydrolytic and oxidative. The hyphae of these "white" rot fungi must secrete two kinds of enzymes, one hydrolytic, for example, a cellulase, the other oxidative. It is not known to what extent, if at all, the oxidases assist in the breakdown of cellulose and the secretion of such enzymes is therefore as yet more a matter of interest than of importance when the decomposition of cellulose is under consideration.

Bavendamm (1928) grew *Merulius lacrymans*, *Coniophora cerebella*, *Trametes radiciperda* and *Stereum purpureum* on agar plates to which test substances had been added. These substances were tannic acid, pyrogallol, hydroquinine, resorcinol, guiacol, phloroglucinol, gallic acid and tyrosine in concentrations ranging from 0·5–0·05%. Within 8 days of inoculation the plates bearing *T. radiciperda* and *S. purpureum* developed a red-brown or dark brown coloration with most of the test substances, the coloration developing not only in the agar with which the hyphae were in contact but to a considerable distance—up to 10 mm.—in advance of the tips of the hyphae. This coloration was interpreted as showing that the fungi secreted into the agar an enzyme of the catechol oxidase type such as produces the black-brown colour on cut surfaces of potato and apple. The shade and intensity of the colour which developed depended chiefly upon the test substance used. The cultures of *M. lacrymans* and *C. cerebella*, however, remained perfectly clear and at no time developed a brown pigmentation. This physiological separation is of great interest, as *M. lacrymans* and *C. cerebella* are "brown" rots attacking cellulose but not lignin, while *T. radiciperda* and *S. purpureum* are "white" rots attacking both lignin and cellulose. It remains, however, to be shown that the catechol oxidase type of enzyme is active against cellulose and lignin or that other oxidases which are active against one or both of these substrates are secreted as well.

Subsequently Lutz (1931), from an examination of the activity of *Polystictus versicolor*, and Nutman (1929) examining *Polyporus hispidus* have reported that they secrete both oxidizing and hydrolyzing enzymes. In the former the enzyme was of the phenolase type while the latter was a peroxidase. Both fungi are in the "white" rot group.

(2) *The activity of whole cells*

It is proposed to consider bacterial and fungal decomposition of cellulose only in so far as it assists in determining the methods and stages by which the molecule is reduced to units of lower molecular weight which are recognizable as sub-units of the cellulose molecule. The studies of the decomposition of plant products by mixed floras of bacteria and fungi, while yielding valuable information concerning the nitrogen requirements of these organisms and of the conditions necessary for the most efficient management of compost heaps, do not yield much information concerning the mechanism of cellulose decomposition. The most important works from this aspect are those in which pure cultures of either bacteria or fungi are used under controlled experimental conditions.

Dubois (1928) divided the bacteria which attack cellulose into three groups:

1. Strictly aerobic forms which are specific to cellulose.
2. Strictly aerobic forms which attack both cellulose and starch.
3. Facultative anaerobes which attack cellulose and grow on ordinary media.

Hutchinson & Clayton (1919) examined the activities of *Spirochaeta cytophaga* which fits into the first group of Dubois' classification. It uses cellulose exclusively and yields among other products a mucilage and a yellow pigment. They described this mucilage as akin to a pectin. The exact identity of the organism is a matter of doubt and it is probable that their cultures contained more than one species. It is clear, however, that whatever their morphological differences the organisms present were all members of the same physiological group. Winogradsky (1929) examined the mucilage produced by the activity of the organism, described it as an oxycellulose which formed a colloidal solution in water and which on hydrolysis with boiling strong acid gave a solution with strong reducing powers. It differed from oxycellulose produced by chemical methods in that it had no power of reducing Fehling's solution. Loicjanskaja (1937) described the mucilage as a polyglycuronic acid and regarded it as the first stage in the decomposition of cellulose. Walker & Warren (1938) made a detailed study of the metabolism of an organism which was physiologically and morphologically identical with *S. cytophaga*. Following Winogradsky's classification they called it *Cytophaga Hutchinsoni*. The organism is most active under conditions of good aeration, in 21 days 16% of the cellulose supplied was decomposed, one-third appearing as mucilage and the remainder as carbon dioxide. The mucilage gave C 40·1%; H 6·7% (cellulose C 44·4%; H 6·2%) equivalent 1107, furfural 6% and uronic acid 14%. Dilute acid hydrolysis resulted in the production of xylose which may have been a degradation product of the uronic acid and a black humin material. It is probable that if this latter fraction had been hydrolysed with 72% H_2SO_4 in the cold, glucose would have been obtained. There is no doubt that this mucilage is an oxycellulose, which contains approximately six glucose units for each molecule of uronic acid. If six glucose units are linked with one uronic acid unit to form a single molecule, the observation of Grassmann, Zechmeister, Toth & Stadler (1933), referred to on p. 24, concerning the molecular size of the substrate in relation to the activity of the oligosaccharase and poly-

saccharase which they isolated from *Aspergillus oryzae* would be of even wider importance. The presence of uronic acid units in the mucilage is of great interest and the significance of their presence will be considered later. The remarks of Walker and Warren in this connexion may be quoted: "It would seem that the primary alcoholic groups of the cellulose molecule are oxidized to carboxyl throughout the length of the chain; that the consequent instability renders easier fragmentation of the large molecule."

Peterson *et al.* (1930) have isolated an aerobic organism from horse dung which decomposes both cellulose and starch. The most interesting feature of its physiological activity is that glucose appears in the fermenting mixture when cellulose is used as a substrate. The hydrolytic products do not contain cellobiose but do contain water-soluble carbohydrate. This organism is typical of Dubois' second group. Scott *et al.* (1930) reported the fermentation of cellulose by a thermophilic bacterium, in form a small, very motile rod. The end products included reducing sugars which were not wholly composed of glucose but did not contain cellobiose. Their explanation of the absence of cellobiose is of interest and would apply equally well to the observations of Peterson *et al.* (1930). Both worked at 55° C., and at this temperature cellobiase is active as well as cellulase. The accumulation of glucose and other carbohydrates in the media is probably the result of the greater activity of the hydrolytic enzymes than of the oxidative enzymes at the working temperature of 55° C. The influence of external conditions in determining the presence or absence of intermediate decomposition products during the bacterial decomposition of cellulose is established by certain observations of Pochon (1934*a*, *b*). He isolated a facultative anaerobic cellulose fermenter, which he called *Plectridium cellulolyticum*, from the stomach of a cow. In culture at *p*H 8 with cellulose as the substrate the products of fermentation included formic, acetic and propionic acids, but no reducing bodies. When the culture was maintained at *p*H 4·8, glucose was isolated from the medium to the extent of 10% of the cellulose fermented.

A number of anaerobic cellulose fermenters have been described, but little is known of the methods by which they attack the substrate. Omeliansky (1902) claimed to have isolated in pure culture two anaerobic cellulose fermenters under the titles of *Bacillus fossicularum* and *B. methanicus*. Khouvine (1923) described a similar organism specific to cellulose and incapable of attacking sugars, *B. cellulosae-dissolvans*. Khouvine & Socters (1935, 1936) found that by suitable treatment this organism could be brought to attack glucose and develop aerobically on agar slopes. Werner (1926) described another organism of the group under the title of *B. cellulosam-fermentans*. The studies of Cowles & Rettger (1931) on the nutritional requirements of the group of obligate, anaerobic cellulose fermenters which they declared were common in soils, muds and faeces suggest the line of their attack on cellulose. These organisms can attack cellulose, starch, dextrin, xylose and arabinose, but are unable to use glucose, glycerol, lactose and many other carbohydrates as sources of energy. Nothing is known of the early stages in the decomposition of cellulose, but the ability to ferment pentoses and not hexoses gives a clue to the later stages. The glucose units in the cellulose molecule may be oxidized to uronic acids, some part

of the medium serving as a source of oxygen, the uronic acids decarboxylated and the resulting pentose or pentosan serving as a substrate for further bacterial attack.

A considerable number of fungi have been described as having the power to attack cellulose. The species which have been most extensively studied are those which are described as moulds typified by *Aspergillus*, *Penicillium* and *Trichoderma* and those placed in the group of *Basidiomycetes*, including species of *Merulius*, *Paxillus* and *Polyporus*.

Reference has already been made to the separation of the timber-rotting fungi into two groups "brown" and "white" rots, p. 25. The nature of the attack by "white" rot fungi on timber has been studied by several workers including Hawley *et al.* (1928), Campbell (1930, 1931, 1932), Campbell & Wiertelak (1935) and Wiertelak (1932).

Campbell (1932), after examining the residues from timber attacked by a number of "white" rots, subdivided the group as follows:

1. Those which attack lignin and pentosans in the early stages, the attack on cellulose being delayed—*Polystictus versicolor*.

2. Those which attack cellulose and its associated pentosan in the early stages and lignin later—*Armillaria mellea*.

3. Those which attack both lignin and cellulose in the early stages, but in varying proportions—*Polyporus hispidus*, *P. adustus*, *Ganoderma applanatum*, *Pleurotus ostreatus* and *Polystictus abietinus*.

The "brown" rots are a much more homogeneous group. The first complete analysis of wood rotted by a pure culture of one of these fungi was reported by Bray & Andrews (1924). Certain features of the constitution of the rotted timber which they observed are now recognized as characteristic results of the activity of members of this group of fungi; i.e. the rapid depletion of the cellulose, particularly the depletion of α-cellulose, and the rapid and early increases in the solubility of the rotted wood in dilute alkali and water. Of particular interest is the fact that the amount of material soluble in dilute alkali rapidly reaches a maximum value from which it falls away with time, suggesting that this fraction is an intermediate degradation product of cellulose which is produced more rapidly in the early stages than it can be utilized, and that subsequently the rate of production falls below the rate of utilization. These observations form the basis of our knowledge of the activities of "brown" rot fungi. They have been confirmed subsequently by Campbell & Booth (1929) using *Trametes serialis*, Barton-Wright & Boswell (1929, 1931), Boswell (1938), Komarov & Filimonova (1937) using *Merulius lacrymans*, and Hawley *et al.* (1928) using *Lenzites striata*. These qualitative results were followed by Boswell (1938) analysing in some detail certain fractions of rotted wood which clearly contained products of the fungal attack. Examination of the cellulose in the rotted wood showed that the α-cellulose content was reduced to a very low level. Further, the determination of the iodine values of the non-α-cellulose fraction of celluloses from pieces of timber rotted to different extents showed that with increased rotting there were an increased number of free reducing groups in the non-α-cellulose fraction. This indicated that the degradation of the cellulose did not

involve oxidation of the reducing groups at the end of the molecules. The residual cellulose was therefore not similar to the oxycellulose resulting from the activity of *Spirochaeta cytophaga*, which possessed no reducing power. The cellulose had, however, been oxidized by the conversion of the primary alcoholic side chains of the glucose molecules into carboxylic groups. Estimation of the resulting uronic acid in samples of cellulose rotted to different degrees revealed that the increased degree of rotting was associated with increased uronic acid content. Indeed, certain unpublished results of the author on the non-α-cellulose part of celluloses from woods rotted to varying degrees show that the molecular weights per reducing group and per uronic anhydride molecule, calculated for each sample of partially rotted cellulose, are of the same order. This suggests that each hydrolytic break in the cellulose molecule is associated with the oxidation of the lateral —CH_2OH group of one of the glucose units in the molecule. This suggestion is supported by the examination of further cellulose degradation products. That fraction of rotted wood which is soluble in dilute alkali contains, among other substances, molecules of relatively small weight which are composed of glucose and uronic acid units. Calculation of the molecular weight for each reducing group present gives a value of 750, which represents a chain of 4–5 glucose molecules. For each uronic acid molecule the molecular weight is 1180–2020, representing a chain of 7–12 molecules. When it is considered that the reducing values were determined on acetyl derivatives and therefore a certain amount of molecular degradation cannot be ruled out, it is reasonable to assume that each chain of glucose molecules, as represented by the free reducing group, contains one uronic acid molecule. Considering the cellulose fraction and that part of the rotted wood which is soluble in dilute soda together, it can be concluded that the fragmentation of cellulose hydrolytically is associated with the oxidation of lateral —CH_2OH groups in the molecule. The extent of the oxidation is such that each fragment broken from the major cellulose molecule contains one oxidized —CH_2OH group, in other words, one molecule of uronic acid.

These observations greatly strengthen the view expressed by Walker & Warren (1938), p. 27, concerning the possible action of uronic-acid formation in facilitating the degradation of cellulose.

While several workers have developed our knowledge of the quantitative changes which are brought about by "white" rots attacking timber, our knowledge of the qualitative changes is small and in no way as extensive as that of the activities of "brown" rots. Campbell (1932) repeats the generally accepted view that the action of "white" rots is oxidative as well as hydrolytic. The demonstration of an active exoenzyme of the oxidase group in *Merulus lacrymans* oxidizing CH_2OH to COOH, however, brings these two groups of fungi much nearer together. It would appear that all timber-rotting fungi excrete both hydrolytic and oxidative enzymes. The separation of "brown" from "white" rots can be explained rather by differences between the relative activities of the two groups of enzymes in different species than by some fundamental distinction between their metabolic processes. It is possible to regard all these timber-rotting fungi as members of a single graduated series.

Consider the groups into which the "white" rots were divided by Campbell (1932), p. 28, *Armillaria mellea* is nearest to *M. lacrymans*, the destruction of lignin being deferred until the attack on cellulose is well under way. Similarly Hawley *et al.* (1928) observed that, during the early stages of attack by the "white" rot, *Polystictus hirsutus*, on timber, cellulose was preferentially attacked and that during the early stages there was little to choose between this "white" rot and the "brown" *Lenzites striata* in the matter of the changes they brought about in wood. The second group, containing *Polyporus hispidus*, is rather further from the "brown" rots in that lignin and cellulose are attacked simultaneously in the early stages. *Polystictus versicolor* is representative of the extreme "white" rot in that lignin is attacked preferentially. The manner in which the "white" rot fungi attack cellulose is unknown and it can only be inferred from the strength of their exo-oxidase enzymes that oxidation of the molecule is probably as important as hydrolysis.

IV. Conclusion

There is evidence, not only in the case of cellulose-decomposing enzymes present in plants, but also of those in the alimentary canal of the snail which have been studied in some detail by Karrer and his school, that the rate at which cell-free extracts of these enzymes attack the substrate is determined, under constant temperature and *p*H, by the physical conditions of the substrate. The most important factor in this group is the degree of dispersion of the cellulose, that is the extent to which the micelles and other molecular aggregates are broken down to free molecules. The higher the degree of dispersion the more rapid is the rate of enzymic attack. It is necessary to distinguish between a high rate of hydrolysis as the result of using a highly dispersed cellulose as a substrate and a high rate of attack when degraded celluloses such as β-cellulose are used. In the former the molecules appear to be intact and the molecular aggregates broken up, while in the latter the molecules themselves have been considerably degraded into smaller units and it is to be expected that sugar end products will be produced more rapidly than when the original molecules serve as a substrate.

While the dispersion of the molecular aggregates of cellulose is an important factor in determining the rate of its enzymic hydrolysis *in vitro*, there is no evidence to show that any biological mechanism exists for the dispersion of the aggregates under natural conditions. It is recognized that the decomposition of cellulose proceeds much more rapidly under the attack of living organisms than it does under optimum conditions *in vitro*, and it may be that this result is achieved not by the initial dispersion of the molecular aggregates by some active agent, but by other means such as high concentrations of and a constant supply of fresh enzyme. These are conditions which have yet to be investigated, as pure preparations of cellulose decomposing enzymes have not been studied.

Karrer and his co-workers have indicated the importance of another physical character of the substrate, namely the arrangement of the molecules and micelles on the surface of the cellulose fibres.

Pringsheim (1912*b*) stated that cellulose is broken down to glucose by two enzymes, cellulase and cellobiase. Recently, Grassmann, Zechmeister, Toth & Stadler (1933) have examined a cellulase and cellobiase from *Aspergillus oryzae* and have shown that they are really a polysaccharase and a oligosaccharase respectively. The smallest cellulose dextrin which the former and the largest which the latter can attack is a cellohexaose of molecular weight about 1000. The possibility that cellobiase is universally an oligosaccharase must be recognized. In this connexion it is of interest to note that Boswell (1938), examining wood rotted by *Merulius lacrymans*, isolated certain cellulose degradation products which had a minimum equivalent weight and probably molecular weight of about 1200. It is known that these degradation products are intermediates in the process of cellulose breakdown and that their accumulation is only a temporary state in the early stages of the rot due to the initial steps in cellulose decomposition proceeding more rapidly than the final steps. It might be considered therefore that these intermediate degradation products represent the end products of a cellulase hydrolysis, the subsequent, slower breakdown, hydrolytic or oxidative, being due to other enzymes. Further Walker & Warren (1938) examined a mucilage formed from cellulose by the action of *Cytophaga Hutchinsoni* and reported that it had an equivalent of 1107. While this has yet to be shown to be the molecular weight, it is a matter with some potential value. As the degradation products in both cases were isolated by virtue of their solubility in dilute NaOH, it might be that this treatment selectively isolated molecules of about the cellohexaose size. That this is not so is shown by the work of Haworth *et al.* (1939) on hydrocellulose, which showed that the upper limit of molecular size of cellodextrins soluble in 0·24 *N* NaOH is many times that of a cellohexaose.

The position of lichenase is uncertain. That it is specific to the one substrate, lichenin, is unlikely in view of the chemical similarity between lichenin and cellulose, and further investigation will probably reveal that it is a cellodextrinase active only on substrates whose molecular sizes lie between certain limits.

That oxycellulose is a product of both bacterial and fungal decomposition of cellulose is of considerable significance. Walker & Warren (1938) confirmed Winogradsky's (1929) view that the mucilage resulting from the activity of *Cytophaga Hutchinsoni* on cellulose was an oxycellulose. Unlike oxycelluloses of chemical origin it had no reducing power, but like them it contained uronic-acid molecules. Similarly the cellulose decomposition products isolated from wood rotted by *Merulius lacrymans* were oxycelluloses and they contained not only uronic-acid molecules but also possessed considerable reducing power (Boswell, 1938). The conclusion that each molecule of degraded cellulose probably contains one uronic-acid molecule strongly supports the conclusions of Walker and Warren (1938) that the oxidation of the primary alcoholic groups of the cellulose chain appears to increase the ease with which the molecule can be divided. The instability of oxycellulose containing uronic acid molecules has recently been demonstrated by Godman *et al.* (1939) who found that treatment with 0·25 *N* NaOH solution decomposed the molecules producing a large range of end products including formic, acetic, lactic and saccharinic acids.

This instability of oxycelluloses increases the significance of their formation during fungal attack on cellulose and may account for the very wide range of decomposition products with no very obvious relation to cellulose which accumulate in decayed wood.

To summarize, a number of plants secrete enzymes capable of hydrolysing cellulose. The complete hydrolysis, cellulose to glucose, requires at least two, a polysaccharase and an oligosaccharase, which have a common lower and upper limit respectively for the molecular size of their substrate. This lies at the level of a cellohexaose. Cellulose degradation among bacteria and fungi appears to be accelerated by oxidation of the primary alcoholic groups of the cellulose molecule, the resulting oxycelluloses being unstable.

V: SUMMARY

1. During the germination of seeds a cellulase is secreted which decomposes the cell walls of the endosperm prior to the attack by other enzymes on the food reserves within the cells. This enzyme system has been isolated from barley malt and extensively studied.

2. Certain bacteria and fungi contain cellulose-decomposing enzymes. There is evidence that in certain cases the decomposition is facilitated by oxidation resulting in the formation of oxycellulose containing uronic-acid molecules.

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ON THE MECHANISM OF PHOTOSYNTHESIS IN PURPLE BACTERIA AND GREEN PLANTS

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(With 6 figures in the text)

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A. Qualitative theory of the assimilation curve in purple bacteria

(i) *The sigmoid assimilation curve*

French (1937*a*) has published an interesting paper on the quantum efficiency of *Streptococcus varians*, a species of Athiorhodaceae. He used infra-red light (852 and 892 mμ) and supplied H_2 and CO_2 but no organic material. In accordance with Gaffron's observation on a related species of bacterium (Gaffron, 1935) it was assumed that the photosynthetic reaction was

$$2H_2 + CO_2 = CH_2O + H_2O. \qquad (1)$$

The assimilation/light intensity curve was found to be of sigmoid type as shown in curve I of Fig. 1. The assimilation/H_2 pressure curve has a normal hyperbolic shape (curve II of Fig. 1)[1] and the same seems to be true of the assimilation/CO_2 pressure curve. This suggests that no time effects are responsible for the sigmoid curve in the case of varying light intensity.

[1] This proves that the two H_2 molecules enter the assimilation reaction in two different reactions following each other.

Time effects can be considerable at 25° C. Generally they consist in a linear increase of the rate of assimilation over nearly 2 hr. In the experiments just mentioned, and also in the experiments of French's Table 2, however, time effects apparently have been avoided. No information is given about time effects at 9·2° C., the temperature at which the quantum efficiency experiments were carried out.

In the only quantum efficiency experiment which French has described in detail light intensity was successively diminished. The time effect mentioned therefore cannot bring about a sigmoid curve, but only a deviation from the normal type of curve in the opposite direction. We therefore take it for granted that the sigmoid curve belongs to the steady state of assimilation.

It has also to be considered whether a secondary light reaction offers an explanation for the sigmoid type of curve. This hypothetical secondary light

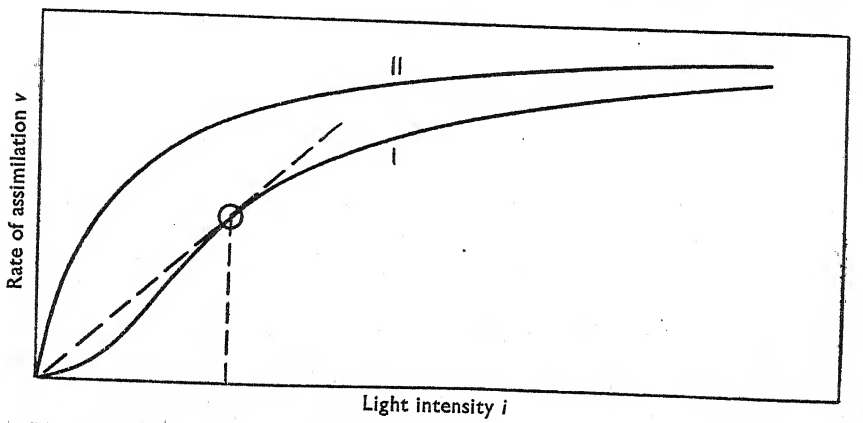


Fig. 1. I, Sigmoid curve; II, Normal curve. ⊙ Point of maximum quantum efficiency or minimum requirement of light quanta.

reaction could lead to a sigmoid curve only if it produced a gas and if the rate of production depended on light intensity. This is illustrated in Fig. 2. Now reactions which produce a gas practically do not occur in the bacteria investigated by French, neither in the dark nor in light; French says "there is in these bacteria no reaction between H_2 and CO_2 in the dark as is the case in some species. The dark readings are very small and in the opposite direction as though there were fermentation or acid production. Only with very dense cultures is this appreciable." In light, if only CO_2 is supplied, there is no gas metabolism according to Table 2 of French's paper. Gaffron (1935) has shown with *Rhodovibrio* that there is also no gas metabolism in light if only H_2 is supplied. Besides, in cases in which an increased dark metabolism may exist in light, namely in *Rhodospirillum rubrum* (French, 1937*b*), and, according to Gaffron (1939) also in *Chromatium* (Eymers & Wassink, 1938), there has not been observed a sigmoid curve but a normal curve with diminished quantum efficiency. Thus we have to conclude that the sigmoid curve is characteristic of the process of assimilation itself.

(ii) *Unstable photo-intermediates*

It has often been pointed out in the discussion of the photosynthesis of green plants that a sigmoid curve would be brought about if the life period of the photo-intermediates were limited (cf. Wohl, 1940, pp. 35–6). This explanation can also be applied to French's curve. To make the meaning of "photo-intermediates" clear the suggested model of the photosynthetic process is described below.

It is most plausible to assume that in photosynthesizing bacteria as well as in the chloroplasts of green plants there is a fixed number n of *reducing centres* where

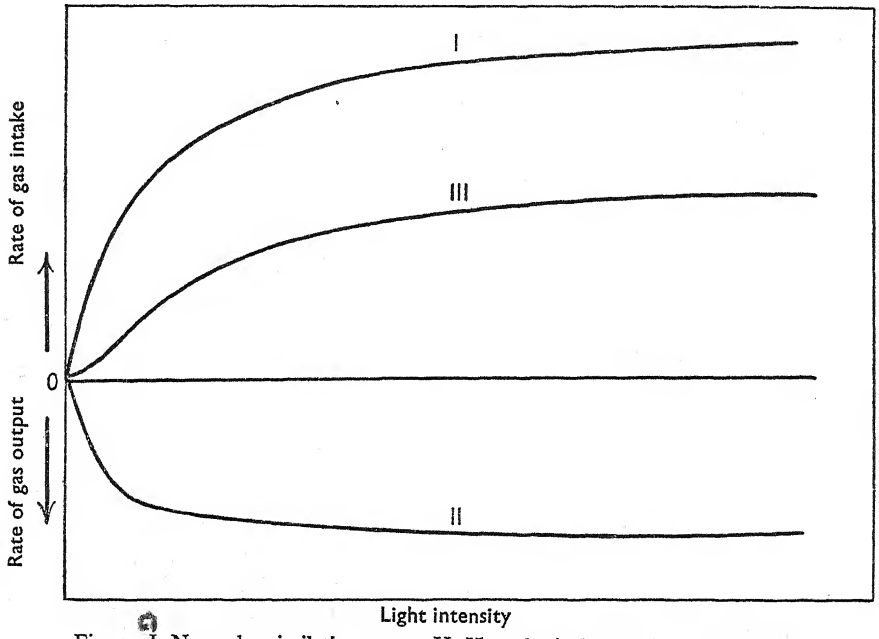


Fig. 2. I, Normal assimilation curve; II, Hypothetical secondary light process; III, Resulting sigmoid curve.

the light quanta absorbed by the pigment are collected—in some form or other—and where the substrates and the energy of the light quanta meet. We have to assume that four light quanta are necessary to bring about the process of equation (1).[1] (From the formal point of view the reducing centre might be identical with one pigment molecule.) Photosynthesis then takes place in *cycles* each consisting of four light reactions (l.r.) together with a sequence of dark processes called collectively the Blackman reaction (Bl.r.). Each cycle thus involves the *initial product* n_0, the *photo-intermediates* n_1, n_2, n_3 and the *photo-product* n_4 which is chemically transformed into the initial product n_0 as shown by the following picture:

$$n_0 \xrightarrow{\text{l.r.}} n_1 \xrightarrow{\text{l.r.}} n_2 \xrightarrow{\text{l.r.}} n_3 \xrightarrow{\text{l.r.}} n_4 \xrightarrow{\text{Bl.r.}} n_0 + \text{products.}$$

[1] Cf. French (1937*a*) and the fundamental investigations of van Niel (1935, 1936) on photosynthesizing bacteria.

n_1, n_2 and n_3, or at least one of them, may be supposed to be unstable on account of the sigmoid shape of the assimilation/light intensity curve. n_4 includes the intermediate stages of the Blackman reaction. We leave it uncertain in what stage the substrate H_2 enters the cycle. CO_2 is supposed not to interfere with the photoreactions (cf. Wohl, 1940), and we must also leave it uncertain for the time being in which way it reacts with the reducing centre (cf. p. 41).

(iii) *Explanation of temperature and pre-treatment effects*

According to French (1937*a*) the concave part of the sigmoid curve is shifted to higher light intensities and rates of assimilation if the temperature is raised. The explanation is that the mean periods of breakdown of the photo-intermediates become shorter at higher temperature. This is to be expected, as the breakdown of these intermediates must be considered as an ordinary chemical reaction.

French further found that the sigmoid character of the curve and consequently the quantum efficiency depends greatly on pre-treatment. This seems quite probable, as the breakdown of the intermediates may depend on enzymatic activities or conditions of the environment which are influenced by pre-treatment.

The number of experiments about pre-treatment is not sufficient to give a clear idea of the state of affairs. In the experiments which have been done, exposure to air in the dark at 25° C. is the most successful means of approaching the normal shape of curve and of increasing quantum efficiency. Almost equally successful is illumination at 25° C. in an atmosphere of CO_2 and H_2 during 10–30 min. preceded by illumination in a CO_2-Argon-atmosphere. No data are given of what the effect is if the preliminary treatment with CO_2 + Argon is omitted.

We should like to suppose that by successful pre-treatment at 25° C. the sigmoid character of the curve *at the same temperature* is diminished. Transfer to 9·2° C. then further reduces the concave part of the curve by the temperature effect mentioned before. Illumination as well as treatment with O_2 may, at high temperature, eliminate factors produced under anaerobic conditions in the dark which facilitate the breakdown of photo-intermediates (cf. the quotation from French (1937*a*) on p. 35).

B. Quantitative theory of the assimilation curves in purple bacteria and green plants

(i) *Constant Blackman period in purple bacteria*

French (1937*a*) showed that with suitable pre-treatment the quantum efficiency in purple bacteria asymptotically approached 1/4, i.e. the value which has also been found in green plants.[1] The full data for the assimilation/light intensity curve are

[1] Eichhoff (1939) has confirmed that the quantum efficiency in *Chlorella pyrenoidosa* is 1/4. Like Warburg he used the manometric method, but suspended the algae in carbonate buffer (85 % $KHCO_3$, 15 % K_2CO_3), thus observing only the oxygen output. Moreover, he measured respiration during 15–30 min. after a preceding period of 30 min.; then assimilation during 15–30 min. after a preceding period of 15 min.; then respiration again during 15–30 min. after a preceding period of 15 min. (and applied light intensities which gave positive apparent assimilation). The objections of Emerson & Lewis (1939) against the results previously obtained by the manometric method do not seem to apply to this procedure. On the other hand, the experiments by Petering *et al.* (1939) and by Magee *et al.* (1939) confirm the lower efficiency found by Manning *et al.*; cf. Wohl (1940).

given only in one case, as was mentioned before. Let us try now to test the explanation of the sigmoid curve given above (i.e. the hypothesis that the photo-process consists of four steps and that the photo-intermediates are unstable) by reproducing this experimental curve theoretically. We shall see that calculation reveals some features which relate photosynthesis in bacteria and green plants. The experimental data are as follows:

Table 1. *Experimental data of French* (1937*a*)

$I \times 10^3$	0	0·139	0·245	0·340	0·43	1·20	∞
h	0	0·20	0·74	(1·26)	1·47	1·88	(2·00)

I = light intensity in cal. per cm.^{2} per min.
h = change of pressure in mm. per 5 min.

According to French (1937*a*) $I \times 10^3 = 1$ corresponds to $i = 1{\cdot}02 \times 10^{14}$ quanta of infra-red light absorbed per cm.^{2} per sec., and $h = 1$ corresponds to $v = 3{\cdot}24 \times 10^{12}$ CO_2 molecules assimilated per cm.^{2} per sec. Extrapolation shows that the maximum rate of assimilation is $h_{max.} = 2{\cdot}0$, and interpolation gives the point of maximum quantum efficiency or *minimum* (*optimum*) *requirement of quanta* $q_{opt.}$ marked in Fig. 1. We find for that point

$$I_{opt.} \times 10^3 = {\cdot}340, \quad i_{opt.} = 34{\cdot}7 \times 10^{12},$$
$$h_{opt.} = 1{\cdot}26, \quad v_{opt.} = 4{\cdot}08 \times 10^{12}.$$

The optimum requirement of quanta in this experiment therefore is

$$q_{opt.} = \frac{i_{opt.}}{v_{opt.}} = 8{\cdot}50$$

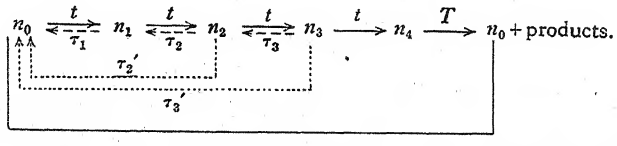
light quanta per CO_2 molecule assimilated.

The theory can be tested in the first place by the ratio of the maximum rate of assimilation $v_{max.}$ to the rate $v_{opt.}$ at the point of optimum quantum efficiency. French's experiment gives

$$\frac{v_{max.}}{v_{opt.}} = \frac{h_{max.}}{h_{opt.}} = 1{\cdot}59.$$

We use the following reactional scheme:

(*a*)



t is the mean interval between the arrival of light quanta at the reducing centre. It equals n/N if N is the number of quanta conveyed to n reducing centres per sec. T is the mean period of the Blackman reaction, τ_1, τ_2 and τ_3 are the mean periods of hypothetical reactions in which the photo-intermediates break down to the products immediately preceding. Finally τ_2' and τ_3' are periods of hypothetical reactions in which the second and third intermediates break down directly to the initial product n_0.

Now, if only one intermediate is supposed to be unstable, i.e. if any one of τ_1, τ_2, τ_3, τ_2' or τ_3' is supposed to be finite, $v_{max.}/v_{opt.}$ cannot be smaller than 2. The same is true if we assume that only two light quanta instead of four are required for equation (1) because there is only one photo-intermediate in this case. If we assume that the periods τ_1, τ_2 and τ_3 are all finite and equal to one another $v_{max.}/v_{opt.}$ can be made equal to 1·59, but then the optimum quantum requirement $q_{opt.}$ is 48 instead of 8·5. If τ_1, τ_2' and τ_3' were finite and equal to one another $q_{opt.}$ in the case $v_{max.}/v_{opt.}=1{\cdot}59$ would be 107 instead of 8·5. If we assume that the life periods of the intermediates differ from one another, the discrepancy becomes still worse. Scheme (*a*) therefore is unable to explain the observed curve. Anyhow, the hypothesis that τ_1, τ_2 and τ_3 are finite can at least be said to give much better results than the alternative one that τ_1, τ_2' and τ_3' are finite. We therefore abandon the latter.

The assumption that the fourth photo-reaction is reversible has quite different consequences. It does not help to produce a sigmoid curve, but it diminishes the quantum efficiency at low light intensity as the reversibility of the preceding steps does too. If the mean period of the back reaction $n_4 \rightarrow n_3$ is called τ_4 and is supposed to be equal to the Blackman period T while τ_1, τ_2 and τ_3 are assumed to be finite and equal to one another as before, the value $v_{max.}/v_{opt.}=1{\cdot}59$ is, by calculation, connected with an optimum requirement of 72·6 quanta compared with 48 quanta in the case $\tau_4=\infty$ and $\tau_1=\tau_2=\tau_3$. Thus it is of no use here to assume reversibility of the fourth photo-reaction.

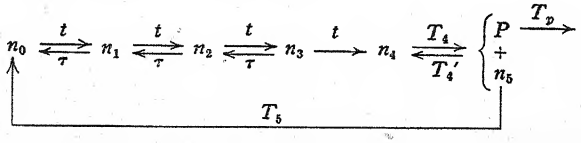
(ii) *Back reaction of the product of assimilation*

(1) *Purple bacteria*

In order to furnish a low value of $v_{max.}/v_{opt.}$ together with a low value of $q_{opt.}$ theory has to provide a factor which slows down further increase of the rate of assimilation at high light intensity. This factor is a lengthening of the Blackman period by high rates of assimilation. It would be brought about e.g. if the product of assimilation were formed by a reversible reaction. For the recombination of the product with the reducing centre retards assimilation. It is negligible at low rates of assimilation where the steady concentration of the product is low and it becomes the more important the more the product is formed.

If we assume, then, that three photo-reactions and the Blackman reaction are reversible, we arrive at the following scheme:

(*b*)



τ is the life period of each of the photo-intermediates. P is the product of assimilation formed by the reaction $n_4=n_5+P$. The mean period of the reaction of formation of P is T_4. The mean period of the reverse reaction is $T_4'V/P$ if V is the volume and P/V is the concentration of P in the steady state. n_5 is transformed into the initial product n_0 after a mean period T_5. The product P disappears by a further metabolic process with the mean period T_p.

This scheme makes a considerable improvement, which however is not sufficient even if extreme assumptions are made. For in the extreme case that the back reaction between P and n_5 is relatively very fast, or to be more exact that

$$\sqrt{\frac{4n\,T_p\,T_4\,T_5}{T_4'V}} \gg T_4 + T_5,$$

the equation arrives at a simple form in which τ and the square root given above are the only constants, while the individual values of the periods concerned with the Blackman process are irrelevant. This equation gives $v_{\max.}/v_{\text{opt.}} = 1{\cdot}59$ with a value for $q_{\text{opt.}}$ which lies a little above 20, while $q_{\text{opt.}} = 48$ was obtained with a constant Blackman period (scheme (*a*)) and the experimental value was 8·5.

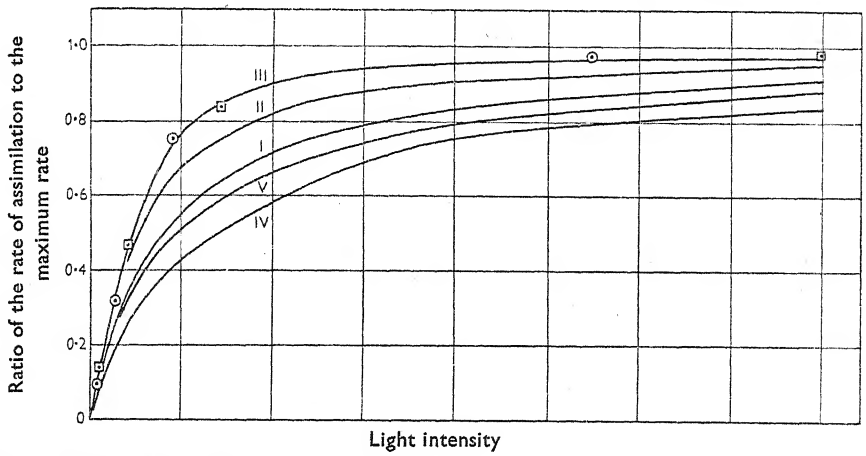


Fig. 3. ⊡ and ⊙, Experimental values by Smith (1937, 1938). I, The normal scheme; II, Scheme (*b*); III, Scheme (*c*); IV, Scheme (*f*); V, The normal scheme with 86·5 % absorption of light. The scales of the abscissa have been chosen individually for every case, so that the initial slopes of the curves are the same. The figure does not show the relative positions of the different assimilation curves, but only allows a comparison of their shape.

(2) *Green plants*

If the life periods of the photo-intermediates are supposed to be ∞, scheme (*b*) can also be applied to green plants. In these, two types of assimilation/light intensity and assimilation/CO_2 concentration curves have been found, curves of "normal" hyperbolic shape found, e.g. by James (1928, *Fontinalis*) under favourable conditions, by Harder (1921, *Fontinalis*), van der Paauw (1932, *Hormidium flaccidum*), Warburg (1919, *Chlorella pyrenoidosa*), Emerson & Arnold (1932, *Chlorella pyrenoidosa*), Emerson (1929, *Chlorella vulgaris*), Emerson & Green (1934, *Gigartina harveyana*), and curves of an "abnormal" shape found by Smith (1937, 1938, *Cabomba caroliniana*), Hoover *et al.* (1933, wheat), Willstätter & Stoll (1918, elm, elder), Wassink *et al.* (1938, *Chlorella vulgaris viridis*). Fig. 3 shows the two types of curves[1]; curve I is hyperbolic, curve III "abnormal".

[1] It may be mentioned that the assimilation/light intensity curves which Smith and also Hoover *et al.* observed at high CO_2 concentrations are a little sigmoid. It does not seem, however, that this interesting point, which might indicate light respiration (cf. Fig. 2) or instability of photo-intermediates, has been proved beyond doubt.

The normal type of curve follows theoretically from the simple scheme on p. 36, in which the Blackman period is constant. The abnormal type seemed (Wohl, 1937 *a*, *c*, 1940) to find an explanation by scheme (*b*). The curve given by this scheme in its extreme form is also shown in Fig. 3. It comes quite near to the abnormal shape of Smith's curve, but the possibilities of this scheme are too limited to give full agreement in this case.

Smith and also Hoover *et al.* found the same shape of curve whether light intensity or CO_2 concentration were plotted as abscissae. Scheme (*b*) too gives the same shape in both cases provided that the change from n_5 to n_0 is specified in the following way, which seems very plausible:

$$n_5 \xrightarrow{T_5} n_0' \underset{-CO_2}{\overset{+CO_2}{\rightleftarrows}} n_0.$$

Here n_0' is the empty reducing centre which takes up CO_2 to form the initial product n_0.

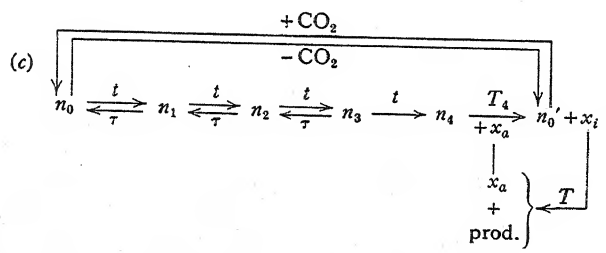
The special form in which scheme (*b*) may be realized in green plants was described by the writer (1937*c*, 1940). It is concerned with the development of O_2, which does not occur in photosynthetizing bacteria. For purple bacteria we have to abandon scheme (*b*).

(iii) *Blocking of enzymes by the Blackman process*

(1) *Purple bacteria*

(*a*) *The main scheme.* There is still another mechanism able to lengthen the Blackman period at high rates of assimilation, which has been used in special forms by Burk & Lineweaver (1935) and Briggs (1935) in connexion with green plants. In this mechanism the Blackman reaction needs an enzyme which is present in only so small an amount that at high rates of assimilation a considerable proportion of it is blocked by the Blackman products. We use this mechanism in the following form:

(*c*)



The Blackman reaction is $n_4+x_a=n_0'+x_i$. x_a is the enzyme in the active form, x_i in an inactive form, perhaps a compound between the enzyme and the product of assimilation. The empty reducing centre n_0' is at high CO_2 concentration quickly and completely transferred into the initial product n_0. x_i after a medium period, T, regenerates the active form x_a. If x_i is the compound mentioned, the product of assimilation is liberated on this occasion. The total amount of enzyme is x; we therefore have $x_a+x_i=x$. The mean period of the Blackman reaction, i.e. of the

transformation of n_4 into n_0', is T_4V/x_a (cp. p. 49); x_a/V is the concentration of the active enzyme.

At low light intensity there is produced so little x_i that x_a is nearly equal to x. At high intensity much x_i is produced, therefore x_a is decreased and the Blackman period T_4V/x_a increased. Now scheme (*c*) is able to give every combination of $v_{max.}/v_{opt.}$ and $q_{opt.}$ down to $v_{max.}/v_{opt.}=1$ and $q_{opt.}=4$. It is even able to do so if we assume that only one of the photo-intermediates has a limited life period. For if T_4V/n, that is the mean period of the reaction of the enzyme with the reducing centres, becomes very short compared with the period of activation of the enzyme T, the curve approaches to the form of the continuous line in Fig. 4. Here quantum efficiency is highest at the point at which the rate of assimilation reaches its maximum value. If T_4V/n is very short and all the photo-intermediates are

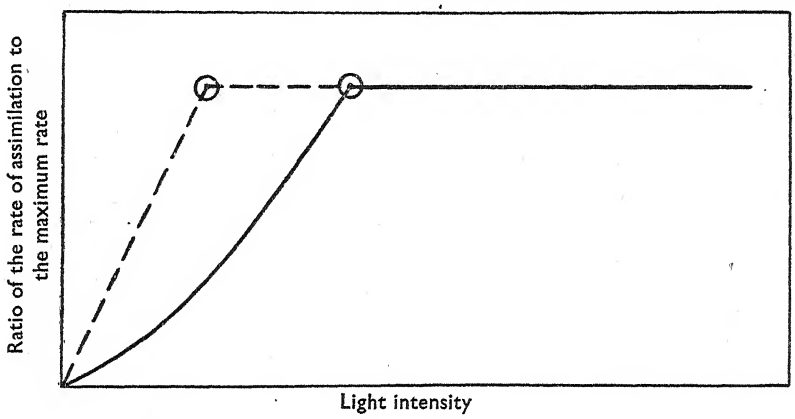
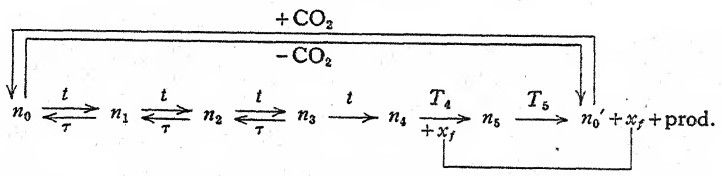


Fig. 4. The extreme case of scheme (*c*). Continuous line: unstable photo-intermediates; dotted line: stable photo-intermediates.

stable, the dotted line in Fig. 4 is valid, which shows a constant quantum efficiency 1/4 or a quantum requirement 4 between $v=0$ and $v=v_{max.}$.

In this scheme the reaction which blocks the enzyme is outside the cycle of processes which the reducing centre undergoes. (Therefore scheme (*c*) does not give any information of the ratio of enzyme molecules to reducing centres.) Burk & Lineweaver (1935) and Briggs (1935) have made this reaction part of the cycle at the reducing centres by assuming that the enzyme is attached at the reducing centre during the Blackman process and released after its completion but exists only in one free form. Thus we have the following scheme:

(*d*)



Here two Blackman reactions occur: n_4 combines with the free enzyme x_f within a mean period T_4V/x_f and the product n_5 on an average is decomposed within T_5 sec.

Obviously we have $x_f + n_5 = x$ if x again is the total amount of enzyme. Now this scheme generally does not possess the full possibilities of scheme (*c*) which are explained by Fig. 4. It does so only if the special assumption is made that the number of enzyme molecules x is very small compared with the number of reducing centres n. (This was assumed by Briggs (1935) for special purposes which we shall have to discuss later on.) For in that case the enzyme can be almost completely blocked by the reducing centres, while the reducing centres are scarcely blocked by the enzyme, just as is the case in scheme (*c*). With $x \ll n$ scheme (*d*) indeed becomes formally identical with scheme (*c*), so that we cannot distinguish between the periods T in scheme (*c*) and T_5 in scheme (*d*) or between x_a in (*c*) and x_f in (*d*). For further discussion we use scheme (*c*) because it implies less extreme assumptions than scheme (*d*).

We have to try now if this scheme, which can describe the relation of the point of optimum quantum efficiency to the maximum rate of assimilation, is able to reproduce the full curve found by French (1937*a*). We have approximated the solution so far as to give $v_{\max.}/v_{\text{opt.}} = 1{\cdot}593$ instead of 1·59 and $q_{\text{opt.}} = 8{\cdot}48$ instead of 8·50. Table 2 as well as Fig. 5 show the agreement of this "main solution" or "solution 1" with the experiment.

Table 2. *Theoretical calculation of French's data of Table 1*

Light intensity $I \times 10^3$	0	0·139	0·245	0·340	0·43	1·20	∞
Rate of assimilation, h							
Observed	0	0·20	0·77	(1·26)	1·47	1·88	(2·0)
Solution 1	0	0·274	0·828	1·26	1·519	1·915	2·007
Solution 2	0	0·384	0·866	1·26	1·535	1·945	2·01
Solution 3	0	0·368	0·856	1·26	1·529	1·925	2·00
Solution 4	0	0·306	0·848	1·26	1·521	1·916	1·991
Solution 5	0	0·294	0·832	1·26	1·529	1·942	2·021

Solution 1: scheme (*c*), or scheme (*d*) with $x \ll n$, main solution, cf. p. 43.
Solution 2: scheme (*c*), or scheme (*d*) with $x \ll n$, additional waste of quanta, cf. p. 44.
Solution 3: scheme (*c*), or scheme (*d*) with $x \ll n$, only one photo-intermediate unstable, cf. p. 44.
Solution 4: scheme (*e*), or general scheme (*d*), cf. p. 45.
Solution 5: Briggs's scheme, cf. p. 49.

Considering the complicated form of the curve the agreement seems satisfactory; perhaps it is within the experimental error. Thus a mechanism operating with four photo-steps and three unstable photo-intermediates can be shown to explain French's curve.

(*b*) *Variations of the main scheme.* As we want to draw some conclusions from the suppositions involved in our solution 1, we have to show how far others can be used in their place. First it was assumed that there is no other waste of light quanta than that provided by scheme (*c*). In other words, it was assumed that all the quanta absorbed by the pigment are conveyed to the reducing centres without loss; or, if we would identify a reducing centre with a pigment molecule, our supposition would mean that *every* pigment molecule is able to act as a reducing centre. Let us now try to give up this hypothesis and to make the assumption that at the point of optimum quantum efficiency only 5·6 quanta instead of the experimental value 8·5

are required by mechanism (*c*), while $v_{max.}/v_{opt.}$ keeps the value 1·59. Then $\frac{8·5-5·6}{8·5} \times 100 = 34\%$ of the absorbed quanta must be wasted before coming into contact with a reducing centre (or 34% of the pigment molecules must be inefficient as reducing centres). This model, approximated up to $v_{max.}/v_{opt.} = 1·595$, gives "solution 2" in Table 2 and Fig. 5. Obviously the agreement is worse than with solution 1. This indicates that the efficiency of the *pigment* in purple bacteria is very

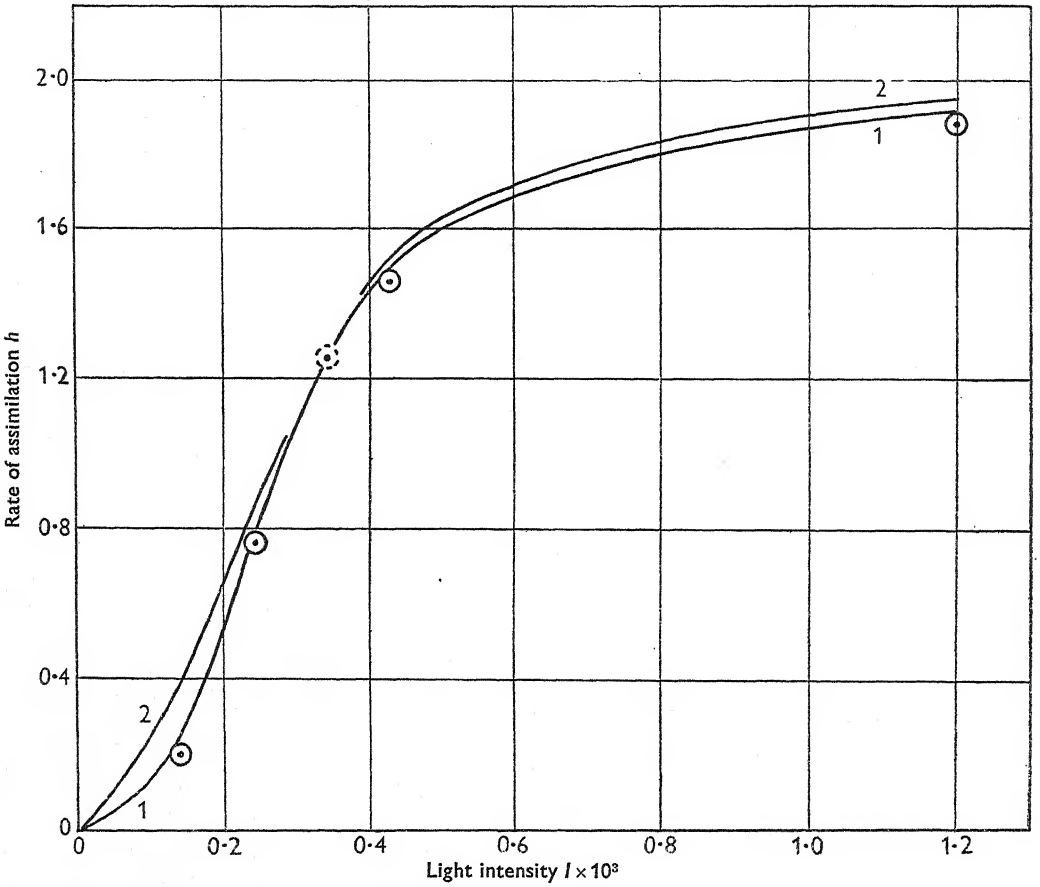


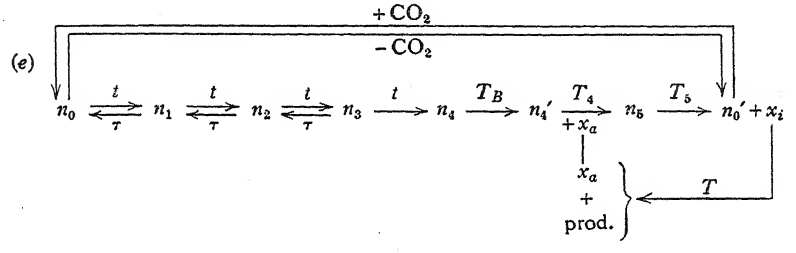
Fig. 5. Graphic representation of the observed values (⊙), solution 1 (curve 1)and solution 2 (curve 2) of Table 2. It was noticed in proof that curve 1 has been drawn slightly too hollow, cf. Table 2.

good even in the case of our Table 1, in which the quantum efficiency was not brought to its highest value by pre-treatment.

A second assumption involved in solution 1 is that all the three photo-intermediates are unstable and have the same life period τ. If we assume that only one of them is unstable—it does not matter which one—solution 3 in Table 2 results. [It is approximated up to $v_{max.}/v_{opt.} = 1·588$ and $q_{opt.} = 8·58$.] Especially at low light intensity, solution 1 is much better than solution 3; this indicates that all the

three photo-intermediates must be unstable. It is not certain, of course, that their life periods are identical, but if they are very different from one another the result resembles the unfavourable solution 3. Thus it is probable that they are of similar magnitude (cf. p. 39).

A third assumption is that in scheme (*c*) the active enzyme x_a reacts with the photo-product n_4 in a collision reaction by which x_i is set free immediately. [It makes no practical difference if it is assumed instead that the enzyme remains attached to n_4 for a very short time before it is released as x_i. Also additional Blackman reactions which have no need of the enzyme x are allowed as far as their mean periods are sufficiently short.] The equivalent assumption for scheme (*d*) is that $x \ll n$. We can reconcile these two conditions if we adopt the following more general scheme:



It is a combination of the schemes (*c*) and (*d*), completed by a Blackman reaction $n_4 \rightarrow n_4'$ (mean period T_B) which is not concerned with the enzyme x. Scheme (*e*) becomes identical with scheme (*c*) or (*d*) if $T_B + T_5$ or if $T_B + T$ are supposed to be 0. From the point of view of this scheme the condition for solution 1 in Table 2 is that

$$\frac{T_5 + T_B}{n} \ll \frac{T_5 + T}{x}. \qquad (2)$$

Condition (2) means that at high rates of assimilation the enzyme molecules are practically blocked by the reducing centres, but not the reducing centres by the enzyme molecules. This condition is obviously fulfilled if $T_B = 0$ and $T_5 = 0$, i.e. if scheme (*c*) holds good, or if $T_B = 0$ and $T = 0$ and $x \ll n$, i.e. if scheme (*d*) holds good in the special form which makes it equivalent to scheme (*c*) (cf. p. 43).

Let us try now to give up condition (2) and to assume e.g. that $\frac{T_5 + T_B}{n} = \frac{1}{4}\frac{T_5 + T}{x}$. This means that we leave scheme (*c*) and that in scheme (*d*), where $T_B = 0$ and $T = 0$, x is supposed to be $n/4$—while in scheme (*e*) x is equal to $\frac{n(T_5 + T)}{4(T_5 + T_B)}$, which cannot be evaluated. The numerical calculation has been approximated up to $v_{\max.}/v_{\text{opt.}} = 1{\cdot}580$ instead of 1·59 and up to the experimental value $q_{\text{opt.}} = 8{\cdot}50$. The figures are given in Table 2 under solution 4. The result is similar to solution 1, but not as good at low light intensity.

A further increase of $\frac{T_5 + T_B}{n}$ compared with $\frac{T_5 + T}{x}$ soon makes the agreement

much worse. With $\frac{T_5+T_B}{n}=\frac{T_5+T}{x}$ schemes (*e*) and (*d*) have both become completely unable to reproduce even the experimental point of minimum requirement of quanta in French's curve, not to mention the course of the full curve. Even if the value of the period T_4V/x is chosen most favourably, the experimental value $v_{max.}/v_{opt.}=1{\cdot}59$ is theoretically coupled with a minimum requirement of 34·7 instead of 8·5 light quanta (while the experimental value $q_{opt.}=8{\cdot}5$ is coupled with $v_{max.}/v_{opt.}=2{\cdot}73$ instead of 1·59). Thus there are distinct possibilities for moderating scheme (*c*) by the assumption of additional Blackman reactions which may include the enzyme *x* or may not, but these possibilities are limited.

A fourth assumption, that the reaction between enzyme and photo-product is irreversible, is unimportant. It makes no essential difference if the reverse reaction is taken into account. It is equally irrelevant for our problem if we consider the reactions $n_4 \rightarrow n_4'$ and $n_5 \rightarrow n_0'$ in scheme (*e*) as being reversible.

(2) *Green plants*

Scheme (*c*), which has proved successful in purple bacteria, is also able to reproduce the "abnormal" shape of assimilation curves found by Smith (1937, 1938) and some other investigators (cf. p. 40) in green plants. For this application, of course, the life periods of the photo-intermediates have to be supposed to be ∞. The agreement obtained with scheme (*c*) is within the experimental error, as shown in Fig. 3. Scheme (*e*), with $\frac{T_5+T_B}{n}=\frac{1}{4}\frac{T_5+T}{x}$ $\left[\text{or scheme } (d) \text{ with } \frac{x}{n}=\frac{1}{4}\right]$, gives almost the same result. With $\frac{T_5+T_B}{n}=\frac{T_5+T}{x}$ scheme (*e*) has become completely unable to reproduce Smith's experimental curve.

The schemes (*c*), (*d*), and (*e*), just as scheme (*b*), are symmetrical with regard to the variables light intensity and CO_2 concentration, i.e. give the same shape of assimilation curve whether the one or the other variable is plotted as abscissae. Only if reaction $n_5 \rightarrow n_0'$, or in scheme (*c*) reaction $n_4 \rightarrow n_0'$, is reversible the symmetry is annulled and the assimilation/CO_2 concentration curve at high light intensity can become similar to the flat curve IV in Fig. 3.

(3) *General remarks*

The lengthening of the Blackman period by increase of the rate of assimilation for different cases is shown in Table 3.

Table 3. *Change of the Blackman period and of the proportion of active enzyme with the rate of assimilation*

	$\frac{T_{low}}{T_{sat.}}$	$\frac{x_{sat.}}{x}$	$\frac{x}{n}$
Solution 1	0·160	0·160	—
Solution 2	0·119	0·119	—
Solution 3	0·065	0·065	—
Solution 4	0·302	$0{\cdot}098_5$	0·25[1]
Scheme (*c*), gr.pl.	0·200	0·200	—
Scheme (*e*), gr.pl.	0·310	0·114	0·25[1]

[1] In the case of scheme (*d*).

The significance of the solutions 1 → 4 is explained under Table 2. "Scheme (*c*), gr.pl." and "Scheme (*e*), gr.pl." means: scheme (*c*) or scheme (*e*) adapted to the abnormal type of curve found in green plants (cf. Fig. 3 and p. 46). It is remarkable that the figures which fit French's curve for purple bacteria are very similar to those characteristic of Smith's curve for green plants. T_{low} means the Blackman period at low light intensity, $T_{sat.}$ the same under conditions of maximum rate of assimilation. The increase is caused, as has been explained before, by a decrease of active enzyme (or free enzyme in scheme (*d*)). The proportion of enzyme which is still active at maximum rate of assimilation is shown under $x_{sat.}/x$. Solution 4 and "Scheme (*e*), gr.pl." give a number for the ratio of enzyme molecules to reducing centres if scheme (*d*) is adopted (cf. p. 45). The solutions 2 and 3 are definitely wrong, as can be seen from Table 2. The most probable solution for purple bacteria lies between solutions 1 and 4 (cf. p. 46).

In the case of normal curves in green plants T_{low} is about equal to $T_{sat.}$ and $x_{sat.}$ about equal to x, apparently because the periods during which the enzyme is blocked are very short (T in scheme (*c*), T_5 in scheme (*d*), T and T_5 in scheme (*e*)).

C. The photosynthetic unit in purple bacteria

The analysis of French's curve supports the suggestion that the photo-reaction determining the rate of photosynthesis at low light intensity consists of four steps both in green plants and in purple bacteria. It is interesting now to compare the maximum rate of assimilation in both classes. For this purpose we use the term "assimilation time" introduced by Willstätter & Stoll (1918), i.e. the time necessary to reduce one CO_2 molecule per pigment molecule at the maximum rate of assimilation. A normal value of the assimilation time in green plants at 25° C. is 30 sec. If we call ν the number of pigment molecules in a sample which gives the maximum rate $v_{max.}$, the assimilation time is $\nu/v_{max.} = 30$ sec. On the other hand, we have $n/v_{max.} = T_{sat.}$, where n is the number of reducing centres and $T_{sat.}$ the Blackman period at maximum rate. If we substitute $v_{max.}$ in the first equation by its value in the second we find

$$\frac{\nu}{v_{max.}} = \frac{\nu}{n} T_{sat.} = 30 \text{ sec.} \qquad (3)$$

In green plants we can replace $T_{sat.}$ by T as the Blackman period is constant, except in the case of "abnormal curves". Emerson & Arnold (1931, 1932), by flashing light experiments, obtained $T \cong 0{\cdot}012$ sec. at 25° C. and $\nu/n = 2500$. The product of both terms is 30, in agreement with equation (3). The high number of chlorophyll molecules per reducing centre has been given the name photosynthetic unit. It has about the same magnitude in a great number of plants (Arnold & Kohn, 1934).

The same calculation cannot be carried out with purple bacteria as they have not been investigated in flashing light. But we can estimate the assimilation time, and the writer feels that this quantity yields an indication whether or not the photosynthetic unit also exists in purple bacteria.

The maximum rate of assimilation $v_{max.}$ in absolute units can be taken from French's paper (1937*a*). It is equal to $6{\cdot}5 \times 10^{12}$ CO_2 molecules reduced per cm.^{2} per sec. (cf. p. 38). A lower limit of the number of bacteriochlorophyll molecules and consequently, according to equation (3), a lower limit of the assimilation time can be estimated from the proportion of the (infra-red) light which was absorbed in French's experiment. The percentage of absorbed light is 34 %. We therefore have $0{\cdot}34 = 1 - e^{-\epsilon\nu}$ or $\epsilon\nu = 0{\cdot}180$, if ν is the number of pigment molecules inside a column of the absorbing liquid of 1 cm.^{2} cross-section and if ϵ is the extinction coefficient expressed in molecules per c.c. as the unit of concentration and cm. as the unit of length. If ϵ' is the extinction coefficient in gram molecules per litre and cm., we have

$$\epsilon = \frac{1000}{6{\cdot}02 \times 10^{23}}\epsilon',$$

and therefore

$$\nu = \frac{0{\cdot}180}{\epsilon} = \frac{1{\cdot}09 \times 10^{20}}{\epsilon'}.$$

According to Stern & Pruckner (1939) and French (1940) ϵ, for bacteriochlorophyll at 852 and 894 mμ., probably is about 50,000. According to the general literature of light absorption by pigments, 100,000 has to be considered as an upper limit. We thus find $\nu \geqq 1{\cdot}09 \times 10^{15}$. The probable value of ν is $2{\cdot}18 \times 10^{15}$.

French apparently used a suspension of 5 mm.^{3} cells per c.c. As in his experiment the layer of liquid was 0·293 cm. thick, we find that within a column of the liquid of 1 cm.^{2} cross-section there were 1·465 mm.^{3} of cells. The molecular weight of bacteriochlorophyll is about 900. Thus 1 c.c. of cells of purple bacteria contains

$$> \frac{1{\cdot}09 \times 10^{15} \times 900{,}000}{6{\cdot}02 \times 10^{23} \times 0{\cdot}001465} = 1{\cdot}1 \text{ mg.},$$

probably 2·2 mg. bacteriochlorophyll. This seems plausible.

Now we find in purple bacteria

$\frac{\nu}{n} T_{sat.} = \frac{\nu}{v_{max.}} \geqq \frac{1{\cdot}09 \times 10^{15}}{6{\cdot}5 \times 10^{12}} = 170$ sec. (cf. equation (3)), probably $\frac{\nu}{n} T_{sat.} \cong 340$ sec.

If we wish to compare this figure with the assimilation time in green plants, we have to reduce it to T_{low} because the value of T_{low} seems to be valid for the whole range of assimilation rates in green plants as was explained before. According to Table 3 and p. 47 T_{low} lies between 0·160 and 0·302 $T_{sat.}$ We use the mean value 0·23 $T_{sat.}$. Thus we find

$$\frac{\nu}{n} T_{low} \cong 77 \text{ sec.}$$

This value refers to 9·2° C. The value of 30 sec. in equation (3) refers to 25° C. It corresponds to 100 sec. at 9·2° C. The values 77 sec. for purple bacteria and 100 sec. for green plants are of the same order of magnitude. Now it seems probable that the reason for the product $\nu/n \times T$ being similar in both cases is that each of the two factors, the Blackman period T and the photosynthetic unit ν/n, have similar values in both cases. Indeed, if the reducing centres were identical with the pigment molecules, i.e. if ν/n were equal to 1, the Blackman period in purple bacteria would have to be ~77 sec. at low and ~340 sec. at high light intensity, which is

extremely improbable. Thus the photosynthetic unit seems to exist also in purple bacteria. More information can only be obtained by experiments with flashing light.

D. Other theories

(i) *Briggs's theory*

(1) *Continuous light in purple bacteria and green plants*

An interesting mechanism has been suggested for green plants by Briggs (1935). It can be understood from scheme (*d*). The mechanism is characterized by five features: (1) The photo-intermediates are of course stable ($\tau = \infty$) [Briggs himself did not subdivide the photo-reaction into several steps]. (2) The number of enzyme molecules is very small compared with the number of reducing centres; this, without feature (4), would make scheme (*d*) equivalent to scheme (*c*). (3) The mean period of the bimolecular reaction of the enzyme with the photo-product n_4 is very small compared with the period T_5; this, without feature (4), would produce the dotted curve in Fig. 4. (4) The last photo-step is reversible, the period of the breakdown reaction $n_4 \rightarrow n_3$ being τ_4 [Briggs assumed instead that "the photo-reaction" is reversible]; this assumption decreases the quantum efficiency at low light intensity and moderates the effect of the assumptions (2) and (3) on the shape of the curve. (5) The reducing centres are identical with the chlorophyll molecules; this feature is at variance with the conception of the photosynthetic unit. Briggs has used his mechanism instead of this conception to explain the experiments of Emerson & Arnold (1931, 1932) with green plants in flashing and continuous light.

The characteristic point of Briggs's scheme is that two extreme assumptions (No. 2 and No. 3) are made, the effect of which is moderated by another assumption (No. 4) which can only be applied to a limited extent because it implies a bad quantum efficiency at low light intensity in conflict with the experimental data. As a result Briggs's scheme is only able to reproduce extreme cases but not the normal ones.

The curve found by French for purple bacteria is such an extreme case. Therefore if we introduce limited life periods of the photo-intermediates into Briggs's scheme, it can be adapted to French's curve nearly as well as scheme (*c*), as is shown by solution 5 in Table 2. Here the back reaction of the photo-product n_4 is half as fast as the forward reaction between n_4 and x. This has the effect that at low light intensity 4·5 instead of four quanta are required for the reduction of one CO_2 molecule.

The shape of Smith's curve found in green plants can also be described by Briggs's scheme. Indeed, the curve of Fig. 3 which is obtained with scheme (*c*) can be obtained with Briggs's scheme if we assume that the back reaction of n_4 is just as fast as the forward reaction between n_4 and x. In that case five quanta are necessary to reduce one CO_2 molecule at low light intensity. [Smith has not determined quantum efficiency.]

In order to describe the normal curves obtained by Warburg and others (cf. p. 40), however, a considerable predominance of the back reaction over the forward

reaction of n_4 and consequently a very bad quantum efficiency had to be assumed, while experiments show that the quantum efficiency is of the order of magnitude of 1/4.

Moreover, in Briggs's scheme the shape of the assimilation curve is not the same with CO_2 concentration as it is with light intensity as abscissa while in a number of experiments these two variables have proved to be formally interchangeable. Indeed, at high light intensity, the moderation of the extreme properties of the scheme by feature (4) does not operate. Therefore, in Briggs's theory, the shape of the rate of assimilation/CO_2 concentration curve under this condition is given by the broken line of Fig. 4. [To avoid this the reaction preceding the combination of the empty reducing centre with CO_2 has to be assumed to be reversible (cf. pp. 46 and 53).]

(2) *Flashing light in green plants*

Briggs's scheme is not in agreement with the data from experiments in flashing light. Fig. 6 shows how the yield of assimilation per flash Δ depends, if the intervals between the flashes are long, on the light intensity of the flashes (*a*) according to the experiment of Emerson & Arnold (1932), (*b*) according to the theory of four photosteps and the photosynthetic unit (Wohl, 1940), (*c*) according to Briggs's theory. In Briggs's theory the maximum yield of assimilation is reached only at extremely high light intensity. Therefore, to adapt this theory to the experiment we have assumed that the "maximum yield" has been reached if by one flash only 4% of the reducing centres (which are identical with the pigment molecules in Briggs's theory) are transformed into the photo-product n_4. At this intensity the yield in reality is only 63·4% of the maximum yield. The comparison is clearly in favour of the theory of the photosynthetic unit.

The dependence of the yield of assimilation Δ on the duration of the dark interval between flashes of high light intensity θ does not follow Briggs's theory either. This theory requires that "the yield for the short periods is greater than expected on the basis of the expression

$$\ln\left(1-\frac{\Delta}{\Delta_\infty}\right)=-\text{const.}\times\theta"$$

(Briggs, 1935; Δ_∞ = yield per flash at very long dark intervals). Arnold (1935), however, has verified this expression—which is the correct one in the theory of the photosynthetic unit—by experiments in flashing light of high intensity.

On the other hand, Briggs's theory requires an induction period which increases when light intensity is decreased, while experiment has proved the contrary. The theory of the photosynthetic unit accounts also for this. Therefore it seems that at present there is no satisfactory alternative to this theory.

The numerical discrepancies in the case of green plants make it also improbable that Briggs's scheme exists in purple bacteria—in spite of the favourable result obtained with solution 5 in Table 2. For we have given indications that the mechanisms in green plants and purple bacteria are similar. In Briggs's scheme,

however, there is no simple transition to the normal mechanism which seems to exist in green plants, as is provided by the schemes chosen in this paper.

(ii) *The theory of Ornstein, Wassink, Reman and Vermeulen (green plants)*

Ornstein *et al.* (1938) have developed a formula which is based on a mechanism capable of serving as a kinetic model of the photosynthetic unit (Wohl, 1937 *b*, *c*, 1940). This formula gives a diminished quantum efficiency at low light intensity.

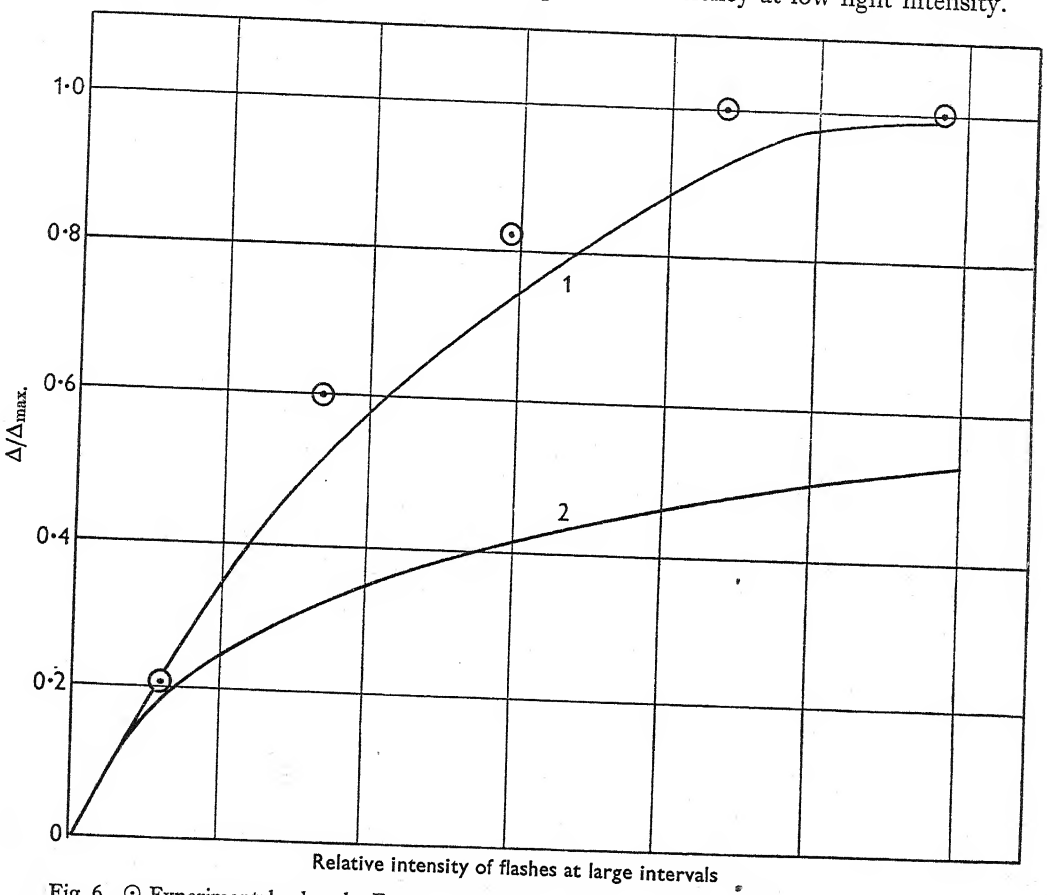


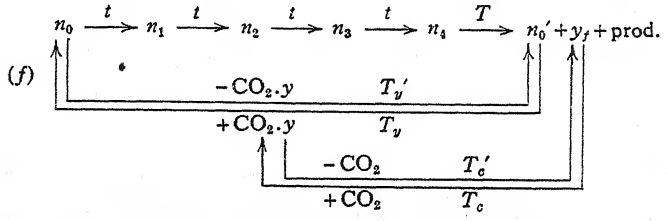
Fig. 6. ⊙ Experimental values by Emerson & Arnold (1932). Curve 1: theory of the photosynthetic unit; Curve 2: Briggs's theory. Ordinate is the ratio of the yield of assimilation per flash, Δ, to the maximum yield, $\Delta_{max.}$. The scales of the abscissae are so adapted that all three curves have the same initial slope.

It is formally identical with the equation derived from Briggs's theory for constant high CO_2 concentration and variable continuous illumination. Therefore it also gives the same result under these conditions as is obtained with scheme (*c*) if in this scheme part of the pigment molecules is supposed to be ineffective (cf. p. 49). In the case of constant high light intensity and variable CO_2 concentration, however, the formula of Ornstein *et al.* gives a normal assimilation curve. Nevertheless, the

theory of Ornstein *et al.* is exposed to the same objection as Briggs's theory, namely, that it requires a very bad quantum efficiency at low light intensity in all cases in which the shape of the assimilation/light intensity curve is more normal than that of the curves found by Wassink *et al.* (1938), Smith and some others (cf. p. 40). Thus it seems that the form of the kinetic model of the photosynthetic unit which was called the "normal" form (Wohl, 1937 *b*, *c*, 1940) (and which is equivalent to the optical model of the photosynthetic unit) is more probable than the modification chosen by Ornstein *et al.* The "normal" form of the kinetic model of the photosynthetic unit provides the simple photo-process which was used in this paper. Therefore, with the help of the Blackman mechanisms of the schemes (*c*)–(*e*), it allows the reproduction of all shapes of assimilation curves between the normal one and the dotted curve of Fig. 3, it allows for a good quantum efficiency at low light intensity in every case, and it gives curves of the same shape with light intensity and CO_2 concentration as variables.

(iii) *Blocking of acceptor molecules by the photosynthetic process*

Radioactive experiments by Ruben *et al.* (1939) with green plants make it probable that CO_2 undergoes some chemical process before it is attached to the reducing centre. It is plausible to assume that CO_2 combines with some "acceptor" and that this compound enters the photosynthetic cycle instead of CO_2. We then can assume that the acceptor is present in only a limited amount as was assumed before for the enzyme (cf. Emerson *et al.* 1940). Thus we have the following scheme:



The free acceptor y_f combines with CO_2 to form the compound $CO_2.y$ (mean reaction period T_cV/CO_2). The empty reducing centre n_0' combines with $CO_2.y$ (mean period $T_yV/CO_2.y$). Both processes may be reversible (mean periods of the reverse reactions T_c' and T_y'). The photo-product is transformed after a mean period T into the empty reducing centre n_0', the free acceptor y_f and the products of assimilation. When the total number of acceptor molecules is y, we have

$$n_0+n_1+n_2+n_3+n_4+y_f+CO_2.y=y.$$

This model gives a good quantum efficiency at low light intensity and high CO_2 concentration only if the number of acceptor molecules y is higher than or equal to the number of reducing centres n and if the reaction between n_0' and $CO_2.y$ is irreversible (i.e. if $T_y'=\infty$). Therefore it cannot be assumed that the number of acceptor molecules is very small compared with the number of reducing centres as has been done in order to replace the theory of the photosynthetic unit (Emerson *et al.* 1940).

The assimilation/light intensity curve at high CO_2 concentration deviates from the normal shape in a sense opposite to the deviation provided by the mechanism discussed before. The reason is that in this mechanism the Blackman period at high CO_2 concentration is not lengthened but shortened by increase of light intensity. For under this condition y_f is instantaneously and completely transformed into $CO_2.y$. Then we have practically only *one* photosynthetic cycle in which the acceptor completely takes part. The time during which the acceptor is blocked by the reducing centre before it is made free again is identical with the duration of the photosynthetic cycle. This duration is the smaller the smaller the interval t between the arrival of quanta, i.e. the higher the light intensity. Therefore, by increase of light intensity, the proportion of acceptor molecules present in the form $CO_2.y$ is increased. Consequently the period $T_y V/CO_2.y$ which is necessary for the transformation of n_0' into n_0 and which forms part of the Blackman period is shortened.

The largest deviation from the normal shape of the assimilation/light intensity curve is obtained if the reaction between n_0' and $CO_2.y$ is irreversible, if the reaction period T is negligible against the period $T_y V/y$, and if y is equal to n. The curve thus obtained is shown in Fig. 3. [Obviously this mechanism—completed by the supposition that the photo-intermediates are unstable—is not able to explain French's curve for purple bacteria.]

If CO_2 is made the variable, we have to consider the *two* cycles of the scheme as we had to do in previous cases and therefore the shape of the assimilation/CO_2 concentration curve is steeper than the normal curve, i.e. similar to Smith's curve (Fig. 3). The feature that the light intensity curve and the CO_2 concentration curve are of opposite type seems interesting, but it is doubtful whether it has been observed.[1]

(iv) *Slow diffusion of* CO_2 *and high light absorption*

So far the velocity of diffusion of CO_2 has been tacitly considered to be much higher than the velocity of the chemical reaction of CO_2 with the reducing centre. If we abandon this assumption but assume that apart from that the mechanism is quite normal, a deviation from the normal hyperbolic shape of curves is also brought about (cf. Maskell, 1928). The rate of the assimilation/CO_2 concentration curve becomes of exactly the same steep type as the assimilation/light intensity and assimilation/CO_2 curve which is produced if the rate of diffusion is very high, but the mechanism is abnormal as provided in scheme (*c*) [cf. Fig. 3 and the experiments by James (1928) and van den Honert (1930)]. The assimilation/light intensity curve, however, tends to become normal with rising CO_2 concentration.

Finally, there is one cause which produces a normal assimilation/CO_2 concentration curve but a slowly rising type of assimilation/light intensity curve. This cause consists in considerable absorption of incident light by the assimilating material (cf. Burk & Lineweaver, 1935). Curve V in Fig. 3 shows how a normal curve is

[1] If in scheme (*e*), (*d*) or (*c*) the reaction preceding the attachment of CO_2 at the empty reducing centre is reversible, opposite types of curves in an inverted sense can be produced, the assimilation/light intensity curve being steeper than the normal one, the assimilation/CO_2 concentration curve flatter (cf. p. 46).

altered if 86·5 % of the incident light is absorbed. In cases like those of French's experiment with purple bacteria where only 34 % of light is absorbed, the effect on the shape of the curve can almost be neglected.

High light absorption can alter an illumination curve of Smith's type so that it approaches a normal type while slow diffusion of CO_2 can alter a normal CO_2 concentration curve so that it approaches Smith's type. [Both circumstances occurring together in a normal case produce, at constant temperature, the characteristic feature of scheme (*f*).] Obviously both circumstances are not responsible for the different shapes of experimental curves gathered on p. 40.

SUMMARY

A scheme for photosynthesis is proposed (scheme (*c*)) in which the product of the photo-process in its narrowest sense reacts with an enzyme in a collision reaction. The enzyme is attached to the photo-product for a very short time only and then is released in the form of a compound with the final product of assimilation (or if there are more than one, with one of them). After some time the enzyme and the product of assimilation separate from one another. The ratio of the mean period of this process of separation to the mean period of the collision reaction mentioned determines the shape of the assimilation curve, which can vary between the normal hyperbolic form and a broken line of the "Blackman type". Further Blackman reactions can be included in the scheme. In every case the shape of curve is the same for the two variables light intensity and CO_2 concentration provided that there are not external circumstances which bring about a difference, and in every case a good quantum efficiency is allowed for at high CO_2 concentration and low light intensity.

Moreover, the scheme is able to reproduce the sigmoid assimilation curve obtained by French for a species of purple bacterium if it is supposed that the three intermediates of the 4-quanta-photo-process are unstable.

It is found by analysis with the help of this scheme that the capacity of one pigment molecule for photosynthesis in continuous light is of about the same magnitude in purple bacteria as in green plants. This makes it probable that the photosynthetic unit also exists in purple bacteria.

Other mechanisms which bring about similar effects and some of which have been suggested as alternatives to the theory of the photosynthetic unit are discussed. No satisfactory alternative seems to exist at present.

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MALE STERILITY IN NATURAL POPULATIONS OF HERMAPHRODITE PLANTS

THE EQUILIBRIUM BETWEEN FEMALES AND HERMAPHRODITES TO BE EXPECTED WITH DIFFERENT TYPES OF INHERITANCE

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(With 2 figures in the text)

The Problem

Male sterile individuals frequently occur in cultures of hermaphrodite plants which have been subjected to inbreeding during the course of genetical experiments. Abortion of the anthers may occur in various degrees from complete abortion of the whole anther to nuclear abnormalities at pollen grain mitosis. Single examples have been reported in *Antirrhinum* species, *Capsella* species, *Lathyrus odoratus*, *Lycopersicum esculentum*, *Oryza sativa*, and *Rubus idaeus* (cf. Lewis, 1940). More than fifteen different types are known in *Zea Mays* (Beadle, 1932). All these are due to the segregation of a single recessive gene. There are two other cases of male sterility in maize due to a single dominant gene (Jones, 1934). Male sterility has not been reported in wild populations of these plants, but presumably it occurs as a rare variant.

Only once has cytoplasmically inherited male sterility arisen as a mutant in cultivated plants, namely in maize (Rhoades, 1933). However, it is the commonest cause of male sterility in species hybrids, where such sterility is often found. In this case there is an interaction of a gene or genes from one of the parental species with the cytoplasm from the other, as in the *Epilobium* hybrids, cf. *Drosophila pseudoobscura* (Dobzhansky, 1935).

The significance of male sterility, apart from its being a starting point for the evolution of unisexuality, is to be found in the "gynodioecious" species. These species exist in two forms, female and hermaphrodite, and the females may constitute a high percentage of the population. But in these plants the natural male sterility is, in no investigated case, due to a single dominant or recessive gene. In *Satureia hortensis*, *Cirsium oleraceum* (Correns, 1928) and *Lolium perenne* (Jenkin, 1931)[1] male sterility is inherited through the cytoplasm. That is to say, the male sterile and the normal hermaphrodite produce only offspring of the same type as the female parent, irrespective of the pollen parent. In *Silene inflata*, *Cirsium pratense* and *Plantago lanceolata* (Correns, 1928), although segregation of

[1] My interpretation of Jenkin's data.

hermaphrodites and females occurs in the progeny of both types, the results do not satisfy any simple genic scheme.

Since genic male sterility is common as a mutation in plants, our problem is to determine why "gynodioecious" species do not show this mechanism of male sterility, and why the remarkable method of cytoplasmic differentiation within a species is found more commonly. The problem can be approached by finding out what conditions are necessary to maintain an equilibrium between females and hermaphrodites in the different types of inheritance. The types of control which will be discussed are: (1) by a single dominant gene, (2) by a single recessive gene, (3) by cytoplasmic inheritance. There are other possible complex mechanisms, as noted in *Silene*, but since they have not been fully analysed they will not be discussed.

(1) *Dominant gene*

When male sterility is due to a dominant gene, the genetic constitution of the female is **Aa** and that of the hermaphrodite **aa**. Females are always backcrossed to the hermaphrodite; the hermaphrodites are either selfed or intercrossed.

Let the proportion of females in the population be p and of hermaphrodites $q\,(=1-p)$, and the fitness of the female relative to that of the hermaphrodite $f(1-p)^x$. The fitness of the females depends on a number of conditions such as the fertility on the female side, optimum hybridity and the efficiency of pollination, and since these conditions are functions of the proportion of hermaphrodites in the population, the term $(1-p)$ must be introduced. We do not know of what power of $(1-p)$ the fitness factor is a function; however, the effect on the equilibrium of varying the power will be discussed after the consideration of cytoplasmic male sterility.

The progeny expected in a stable population is given in Table 1.

Table 1. *The progeny expected in a stable population when male sterility is due to a single dominant gene.* $f(1-p)^x$ *= the fitness of the female relative to that of the hermaphrodite*

	Female	Hermaphrodite
Proportion in population	**Aa**, p	**aa**, q
Female gametes	**A**, $pf(1-p)^x$ **a**, $pf(1-p)^x$	**a**, $2q$
Male gametes	—	**a**, $2q$
Zygotes	**Aa**, $pf(1-p)^x$	**aa**, $pf(1-p)+2q$

Let us consider the relationship between p and f. From Table 1,

$$pf(1-p)^x = pK \quad \text{and} \quad pf(1-p)^x + 2q = qK,$$

where K is a constant. Then

$$2pf(1-p)^x + 2q = f(1-p)^x(p+q).$$

Since $p+q=1$,

$$2pf(1-p)^x + 2 - 2p = f(1-p)^x.$$

Therefore

$$p = \tfrac{1}{2} - \frac{1}{f(1-p)^{x-1}}.$$

This gives a simple relationship between the proportion of females in the population and the fertility of the females relative to that of the hermaphrodites.

(2) *Recessive gene*

When the gene causing male sterility is recessive, the genetic constitution of the female is **aa**, of the hermaphrodite either **Aa** or **AA**. If p is the proportion of **aa** plants, q the proportion of **Aa** plants and $r\ (=1-p-q)$ the proportion of **AA** plants in the population, and $f\,(1-p)^x$ is the same as before, then the expected progeny, assuming random mating of the hermaphrodites, is:

$$\mathbf{aa} \qquad q\,[2pf\,(1-p)^x+q]=pK. \tag{I}$$

$$\mathbf{Aa} \qquad q\,(q+2r)+(q+2r)\,[2pf\,(1-p)^x+q]=qK. \tag{II}$$

$$\mathbf{AA} \qquad (q+2r)^2=rK. \tag{III}$$

$$p+q+r=1.$$

By summation of (I), (II) and (III),

$$(p+q+r)\,K=[2q+2r]\,[2pf\,(1-p)^x+2q+2r].$$

Substituting $(1-p)$ for $(q+r)$,

$$K=(2-2p)\,[2-2p+2pf\,(1-p)^x],$$

$$K=4-8p+4pf\,(1-p)^x+4p^2-4p^2f\,(1-p)^x. \tag{IV}$$

By summation of (II) and twice (III),

$$(q+2r)\,K=2\,(q+2r)^2+q\,(q+2r)+(q+2r)\,[2pf\,(1-p)^x+q],$$

$$K=2\,(q+2r)+2q+2pf\,(1-p)^x,$$

$$K=4q+4r+2pf\,(1-p)^x.$$

Substituting $(1-p)$ for $(q+r)$,

$$K=4-4p+2pf\,(1-p)^x.$$

From (IV),

$$4-4p+2pf\,(1-p)^x=4-8p+4pf\,(1-p)^x+4p^2-4p^2f\,(1-p)^x.$$

Therefore

$$p=\tfrac{1}{2}-\frac{1}{f\,(1-p)^{x-1}}.$$

This is the same relationship between p and f which we obtained for a dominant male sterility gene. By assigning different values of f, a series of values of p can be obtained, thus when $x=0$,

$f=$	1	2	3	4	5	6	∞
$p=$	$-\infty$	0	$\frac{1}{4}$	$\frac{1}{3}$	$\frac{3}{8}$	$\frac{2}{5}$	$\frac{1}{2}$

The relationship is represented graphically in the continuous lines of Fig. 1. It is evident that unless the female is at least twice as fertile as the hermaphrodite, no equilibrium can exist between them except that maintained by recurrent mutation.

The theoretical limit of p is 50 % of the population in all cases. This limit is attained when the fertility of the females relative to that of the hermaphrodites is infinite; this could only be brought about by complete female sterility of the hermaphrodites, viz. maleness.

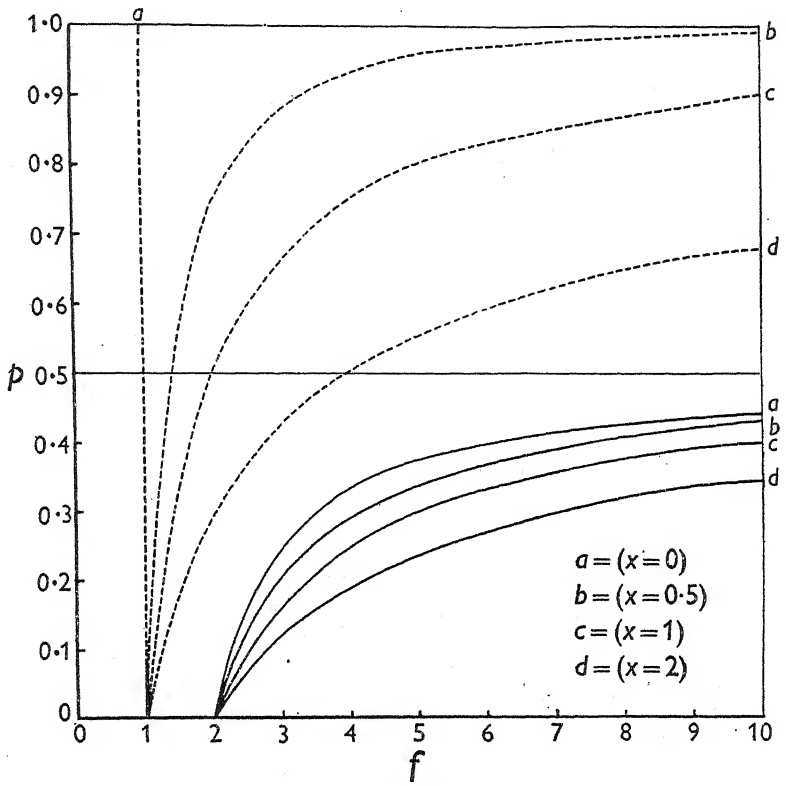

Fig. 1. The relationship between the proportion (p) of females in the population and the relative fertility (female/hermaphrodite) (f). Continuous lines refer to dominant or recessive genes; broken lines to cytoplasmic determination.

(3) *Cytoplasmic inheritance*

In the case of inheritance of male sterility through the cytoplasm, females produce only female offspring and hermaphrodites only hermaphrodites. If the female has only a slight reproductive advantage, say 0·001 over the hermaphrodites, it will spread in the population. The increased fertility of the females may be due to the selective advantage of being outbred, and/or to the reproductive economy resulting from the lack of pollen production. So long as these advantages are maintained, the females will increase in the population until the available pollen of the hermaphrodites is fully utilized. At this point an equilibrium will be reached depending upon the selective advantage of being outbred and the efficacy of pollination, both of which in turn depend upon the proportion of hermaphrodites in the

population. Thus the fitness factor of the females can be conveniently formulated as in the genic cases as $f(1-p)^x$.

Let p be the proportion of females and $q\,(=1-p)$ the proportion of hermaphrodites. Then, for an equilibrium to be maintained,

$$p=pf(1-p)^x, \qquad \therefore \quad f(1-p)^x=1.$$

This is the relationship between p and f which is plotted for different values of x in the broken lines of Fig. 1. The curves show that p has a positive value for any value of f above 1, and that the asymptote is $p=1$ for all values of x. Thus the value of f at which the curve starts and the asymptote are independent of x in both cytoplasmic and genic male sterility. The only effect of x on the equilibrium curves is to change the shape of the curve. As the value of x increases, the steepness of the curve diminishes. The true value of x probably lies between 0·5 and 2·0; this value will be partly determined by the amount of pollen available for the females. When adequate pollination of a large number of females by a smaller number of hermaphrodites is possible, x will be small and consequently the curve will rise steeply. However, the effect of x is slight and does not change the main theoretical relationship between p and f.

DISCUSSION

The results of the equilibrium calculations for a recessive and dominant gene and cytoplasmic inheritance of male sterility show several striking differences. The relation between the fertility of females and the equilibrium in the population is the same for a dominant or recessive gene, and in these cases no equilibrium can exist unless the females are more than twice as fertile as the hermaphrodites. This is a very high value and one that is not likely to be attained in nature at a single stroke, as it would need to be to get a start at all. In contrast to this, if the inheritance is cytoplasmic an equilibrium can exist when the fertility of the female is only slightly above that of the hermaphrodite. Furthermore, the cytoplasmic curves rise more steeply than the genic curves, showing that an increase in fertility of the females in a new environment leads to a greater increase in the equilibrium point in cytoplasmic than in genic male sterility. Another difference is found in the maximum value of p; for the dominant and recessive gene the maximum is $\frac{1}{2}$, and for the cytoplasmic it is 1.

We see, therefore, that cytoplasmically inherited male sterility is admirably suitable for maintaining a balance between the proportion of females and hermaphrodites in the population. If the female had only a slight selective advantage, it would spread in the population until this advantage had been lost by the attainment of the hybridity optimum (cf. Darlington, 1936) and lack of effective pollination. On the other hand, male sterility due to a simple dominant or recessive gene cannot spread unless the female has a very high positive selective value at the start. This does not imply that other genic mechanisms will not be found; other mechanisms are in fact undoubtedly present, as is shown by the results on *Plantago lanceolata* and *Cirsium pratense*. However, before these can be discussed in terms of population equilibria, more data are required.

The significance of male sterility in "gynodioecious" species is in the increased outbreeding that results from it (Mather, 1940). It is an outcrossing mechanism which is extremely sensitive to changes in the hybridity optimum of the population. Since there is a possibility of self-fertilization in the hermaphrodites, these will be more inbred than the females, in the absence of cross-fertilizing genetic mechanisms. If the hybridity optimum has not been attained, an increase in the proportion of females in the population will result; if the optimum has been exceeded, then the females will have a lower reproductive value and will consequently decrease. The sensitivity of the adjustment to the hybridity optimum depends upon the steepness of the curve. This is greatest in the case of cytoplasmic male sterility with adequate pollination of the females, and lowest in the genic male sterility. Again, a change in the hybridity optimum sufficient to produce male sterile plants with double the female fertility of the normals is not likely to arise suddenly; therefore genic male sterility is not adaptable to changes as the cytoplasmic male sterility is.

Cytoplasmic male sterility is an ideal method of maintaining an equilibrium between females and hermaphrodites, and also a sensitive method of adjusting the amount of outbreeding to the hybridity requirements. Simple genic male sterility fulfils neither of these functions and thus its absence in gynodioecious species is not surprising.

Our knowledge of the frequency of male sterility in plant populations and the extent of cytoplasmic differentiation as a cause of it is extremely meagre. These frequencies, which are of considerable importance from the point of view of population genetics, can be determined (1) by counting wild populations, and (2) by examining seedlings derived from seed collected from the wild.

The progeny to be expected on the three types of inheritance are given in Fig. 2. The only complication is in the case of a recessive gene where, as there are two genetically distinct hermaphrodites, some plants will segregate both types while others will not. This complication makes it advisable to collect seed from a number of different females and hermaphrodites and to grow the seed from each plant separately.

Very little is known about the origin of cytoplasmic differentiation within a species. It can arise by a cytoplasmic change analogous to gene mutation, e.g. in *Epilobium* (Michaelis, 1937) and *Zea Mays* (Rhoades, 1933). Cytoplasmic mutations appear to occur more rarely than gene mutations; however, it is probable that changes in the cytoplasm arise more frequently than the data reveal, but are not observed because the effects are so slight. A change producing a large effect such as male sterility is undoubtedly a rare occurrence, but if it does occur in species where increased hybridity is an advantage, it will be immediately selected. Cytoplasmic differences may also arise by the introduction of cytoplasm from another species by interspecific hybridization. If the hybrid is male sterile, it will retain its particular cytoplasm, but by the necessary backcrossing it will eventually have a nucleus of one species; this has been shown in *Epilobium* hybrids (*loc. cit.*). Thus the male sterile form of a species may differ from the normal hermaphrodite by having the cytoplasm of a closely related species.

Interspecific hybrids provide an adequate basis for cytoplasmic differentiation, but the survival of differences is hampered by the genic sterility which so often accompanies the crossing of species. Mutations arising in the cytoplasm of a pure species are rare, but not being accompanied by sterility have more chance of survival.

The New Zealand species of *Hebe* are particularly suggestive in this connexion. Male sterility occurs in a number of species (Frankel, 1940), but at present the mechanism of inheritance is not known. If the mechanism is cytoplasmic, the very

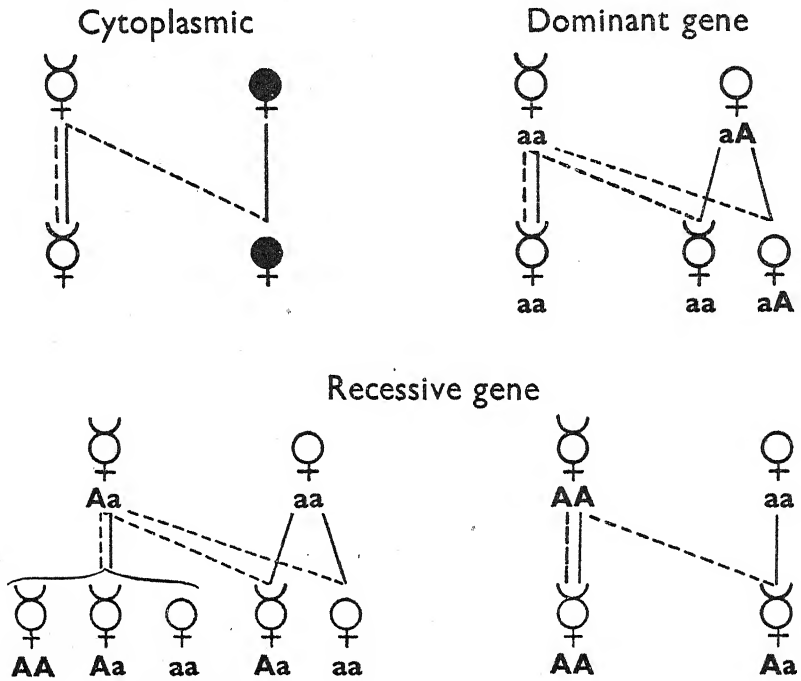

Fig. 2. Progeny expected from natural seed collected from the wild from female and hermaphrodite plants with different types of inheritance (● = cytoplasmic difference, ---- = male gamete, —— = female gamete).

frequent natural interspecific hybridization in the genus (cf. Allan, 1940) should repay investigation.

Cytoplasmic male sterility is unique in being the only observable cytoplasmic difference within a species which is known to exist in wild populations; it is thus good material for determining the origin of extra-nuclear differences.

SUMMARY

1. Male sterility due to a recessive gene is common as a mutant in inbred plants, but is not found in species which are naturally polymorphic for male sterility. In all the "gynodioecious" species fully investigated, male sterility is inherited through the cytoplasm.

2. From the relationship between the proportion of females in the population and their fertility relative to that of the hermaphrodites, I have shown that where male sterility is due to a dominant or recessive gene the females cannot exist in a wild population unless they are more than twice as fertile as the hermaphrodites on the female side. In the case of cytoplasmic male sterility only a slight advantage of the females is necessary.

3. Cytoplasmic male sterility is an outbreeding mechanism particularly sensitive to changes in the hybridity requirements of a species.

I am much indebted to Dr K. Mather for advice on the preparation of this paper.

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THE SIGNIFICANCE OF CERTAIN MORPHOLOGICAL VARIATIONS OF COMMON OCCURRENCE IN FLOWERS OF *PRIMULA*

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(With 23 figures in the text)

VARIOUS species of *Primula* and their derivatives, notably *P. acaulis* (Primrose), and strains of polyanthus are well known to exhibit under cultivation sporadic variations departing in numerous ways from the isomerous pentamerous type. Although more often noticed in the above-mentioned familiar forms, many of these variations are widespread through the genus, and in several of the other species examined (e.g. *P. malacoides*) were frequently observed. At present we are without precise knowledge of the conditions under which these variations arise. It can, however, be said that a study of the adjustments in the anatomical relations associated with these variations has definitely advanced our knowledge of the conditions underlying their appearance and has made it possible to formulate some generalizations with regard to them. *How* they come about has, in fact, supplied a partial answer to the question *why* they come into being.

In treating the various morphological constructions here considered it will be advantageous, owing to the intimate anatomical relations existing between the outer floral whorls, to deal with the perianth and androecium together and separately from the gynaeceum.

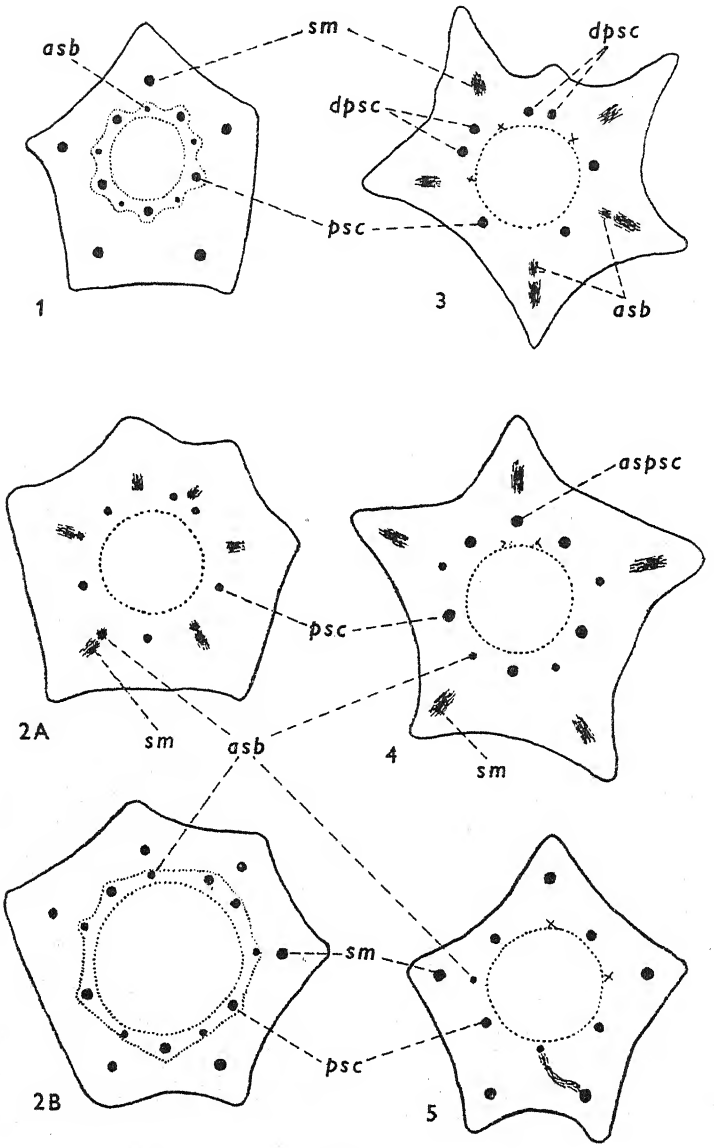
1. The perianth and androecium

Morphological variations occurring in the whorls of the perianth and androecium fall naturally into two categories, viz. those resulting from a fundamental change of ground-plan and those due to modifications superimposed upon the typical ground-plan. In order to make this distinction clear, it will be advisable at this point to recall certain structural features of the normal *Primula* flower.

In *Primula* the arrangement of the sepals is quincuncial. This arrangement is constant. It leads inevitably to the setting up of unequal spatial conditions on the several radii. The arrangement of the petals, on the other hand, is extremely variable. Examination of 110 corollas from an individual plant of *P. malacoides*, for example, yielded the following numbers: quincuncial 57, cochlear 21, vexillar 16, convolute 10, imbricate proper 6. A count of 128 corollas from a bunch of wild *P. elatior* flowers yielded 84 quincuncial, 28 cochlear, 12 vexillar and 4 convolute. These variations in the scheme of petal overlap evidently depend upon conditions existing outside the corolla and do not appear to stand in any direct relation to the structural

departures from type here described. But there is little doubt that the different conditions obtaining on the several construction radii as a result of the *sepal* arrangement offer a starting point for some heteromerous constructions, while contact pressure during early stages in development may possibly be another factor contributing to this kind of asymmetry. It may be noted in this connexion that in the section Vernales (e.g. oxlip, primrose, cowslip, with their polyanthus derivatives, and *P. Juliae*), in which the sepals are without separate marginal vascular systems of commissural origin (see Fig. 16 B) and the midribs form strongly projecting ribs so that the flower base is five-angled in cross-section, two of these angles are distinctly nearer together than the others (see Figs. 1, 3, 4, 5, 8, 9, 11 A, 13 A, 13 D, 16 A). Similar unequal spacing is also often, but not invariably, characteristic of the hexamerous calyx (see Figs. 2, 13 B, 13 C). For example, the cross-section of the extreme base of a six-merous flower of polyanthus is generally bluntly pentagonal as in the pentamerous flower, a sixth angle, corresponding with the sixth sepal, only appearing a little later in such a position that the interval between this sepal rib and its neighbour on one side, or sometimes on both, is less than that between the other sepal ribs. In accord with this late development the corresponding antesepalous staminal bundle becomes detached later than the other five from its antesepalous trunk cord.

The trunk cords furnishing the midrib bundles of the sepals turn out from the central cylinder on the corresponding set of radii, those giving rise to the petal midribs originate similarly on the alternate set. In all *Primula* spp. so far investigated a bundle is ordinarily detached from the inner face of each of these sepal cords below the exsertion level of the calyx. These detached bundles run a straight course up the corolla-androecium tube, alternating with the petal cords (Figs. 6, 7) and eventually forking shortly below the level of separation of the corolla lobes (Figs. 6, 10, 11 B, 12, 19). As this dichasial branching takes place the main bundle comes to an end, while each fork enters the adjacent petal lobe and there gives rise to a marginal system separate from the midrib system or sometimes anastomosing with it at one or two points (Figs. 10 A, 11 B, 19). As has been shown in earlier accounts these detached bundles are those of an antesepalous staminal whorl, the members of which do not ordinarily attain separate morphological form in *Primula* as they do in a few other genera (e.g. *Samolus* and in a lesser degree *Soldanella*) (Saunders, 1932, p. 264; Saunders, 1934, pp. 131–139; Saunders, 1939, p. 206; Saunders, 1940, pp. 107–109). But one species, *P. floribunda*, affords a striking exception to the general rule. Under cultivation this species may produce numerous flowers showing a whorl of petaloid structures alternating with the petals (Fig. 14). These structures vary considerably in size. They may take the form of small entire lobes between the larger petal lobes or they may attain the size of the petal lobes, resembling them so completely in size and form as to give the appearance of a second corolla. The antesepalous staminal bundles, which in such flowers are prolonged as main bundles above the level of origin of the primary laterals entering the adjacent petals (monopodial branching), are continued into these petaloid structures and there undergo further branching (Figs. 14 C, 14 D) or only these later



Figs. 1–5.

laterals may be formed (Fig. 14 D). It is clear that these structures represent the members of an antesepalous staminal whorl modified into petaloid staminodes.

The petal cords, unlike those on the sepal radii, vary in constitution in different species. In the majority a pair of bundles is detached from the outer face of each cord below the level at which the calyx is exserted. The two bundles thus derived give rise to a marginal system of commissural origin in the neighbouring sepal on each side (Figs. 17 B, 18 A). In other species (e.g. those of the Vernales group among others) these bundles and the marginal systems to which they give rise are lacking (see Fig. 16 B). But in all species a bundle is detached from the inner face of these cords immediately below the level at which the stamens which they serve spring from the corolla-androecium tube (Fig. 18 B), the petal and fertile stamen whorls being always isomerous and superposed in the normal flower. As a rule the petal midrib bundle ceases below the petal apex and is overtopped by its own laterals as well as by the above-mentioned antesepalous staminal system. In accord with this interrelation the petal apex is retuse or deeply bilobed (Figs. 10, 11 B, 12, 14, 19),

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Legends of Figs. 1–5

Figs. 1–5. Transverse sections of the perianth-androecium ring (semi-diagrammatic). Figs. 1, 2. Polyanthus. Fig. 1. From a typical, pentamerous flower bud. Fig. 2. From a flower of the form K6 C6 A5 antesepalous staminal bundles+6 antepetalous stamens. A. At the level at which the antesepalous staminal bundles are becoming detached from the sepal cords. B. After these bundles have become detached. The sepal cords are unequally spaced owing to the development of a sixth cord (right, back). No antesepalous staminal bundle is detached from this latter cord owing to the close juxtaposition of the two adjacent petal cords. Figs. 3–5. *Primula acaulis*. Fig. 3. From a flower of the form K5 C7 through duplication of two petals A2 full-length and 3 short-length (indicated by ×) antesepalous staminal bundles+7 antepetalous stamens. The short-length bundles occur on the sepal radii lying between, and next to, the two duplicated petals. Fig. 4. From a flower of the form K5 C6 through the formation of an additional petal superposed on one of the sepals (centre back). A4 full-length and 1 short-length (indicated by > <) represented by the two lateral branches in the adjacent petal lobes, the main bundle running normally up the corolla-androecium tube being absent owing (doubtless) to the close juxtaposition of the petal cord on this radius and those of its two neighbours. Fig. 5. From another flower of the form K5 C4 through absence of a petal on the radius between the two sepals lying nearest to one another (front) A2 full-length and 2 short-length antesepalous staminal bundles+4 antepetalous stamens. To balance the absence of a petal-stamen cord between the two front sepals and restore vascular symmetry the antesepalous staminal bundle detached from one of these sepal cords (front right) deviates from a straight course to the position proper to the missing petal-stamen cord. This compensating adjustment probably accounts for the non-detachment of an antesepalous staminal bundle from the other front (left) sepal.

Since the explanatory lettering to the different figures is the same in all, the full list is given below in order to avoid repetition.

a, anther.
apb, antepetalous staminal bundle.
apf, antepetalous staminal filament.
as, antesepalous staminode.
asb, antesepalous staminal bundle or system.
aspsc, antesepalous petal-stamen cord.
cm, commissural marginal vein.
dasb, duplicated antesepalous staminal bundle.
dpsc, duplicated or double petal-stamen cord.
dsf, duplicated or double staminal filament.
dpnr, duplicated or double petal midrib.
dsm, duplicated or double sepal midrib.
fcb, fertile carpel bundle.
p, petal.
pl, petal lateral bundle.
pm, petal midrib.
psc, petal-stamen cord.
pt, parenchymatous tract.
r, radius.
rpt, ruptured parenchymatous tract.
rvtg, residual vascular tissue not yet organized into carpel bundles.
s, space.
sasb, short-length antesepalous staminal bundle.
sc, sepal cord (i.e. sepal midrib and antesepalous staminal bundle).
scb, sterile carpel bundle.
sf, cut base of stamen filament.
sg, stigma.
sm, sepal midrib.

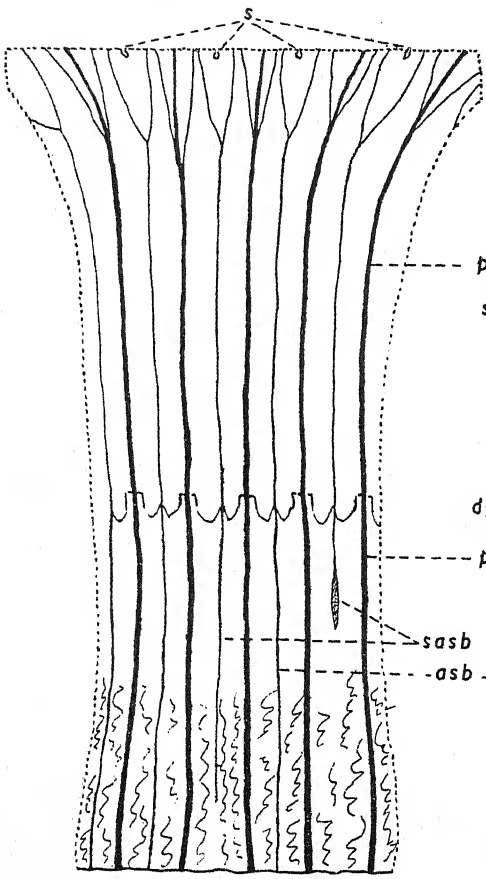


Fig. 6.

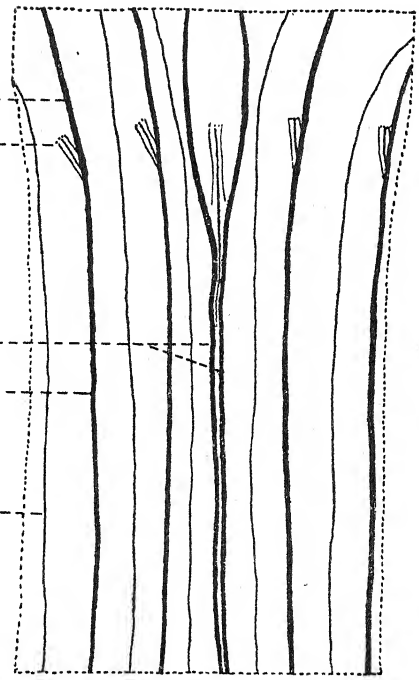


Fig. 7.

Fig. 6. Polyanthus. A pentamerous corolla-androecium tube split lengthwise and opened out showing two antesepalous staminal bundles in process of disappearing; one makes its appearance a short distance from the base of the tube, the other arises from a clump of tracheides at a higher level. In the basal region of the tube the parallel longitudinal files of parenchymatous cells have undergone distortion forming sinuous tracts through imperfect co-ordination in the amount or/and rate of extension of this tissue and of the vascular bundles. At the top of the tube the nicks in the outline (indicated by *s*) show the points at which the several petal lobes begin to separate.

Fig. 7. *Primula kewensis*. Part of the corolla-androecium tube from a flower of the form K 5 C 6 (through the break-up of a double petal-stamen cord into two separate petal midribs) A 5 antesepalous staminal bundles + 5 fertile stamens. The separation of the two petal midrib bundles and the springing free of the stamen filament occur at about the same level, but the undifferentiated stamen strand can be traced downwards for some distance between the two petal components of the cord.

whereas it is convex and entire when the midrib overtops the secondary veins as occurs in some other genera (e.g. *Cortusa* and *Samolus*).

We may now examine the manner in which the normal anatomical relations outlined above are adjusted in flowers showing certain commonly occurring morphological variations from the type. Although most of the exceptional constructions described were observed in polyanthus and primrose, these forms being most readily available, it must be understood that when similar variations occur in other species and genera the same adjustments are made, and hence that the conclusions reached are generally applicable to the whole family.

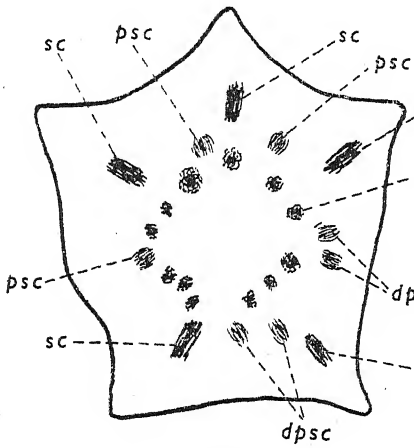


Fig. 8.

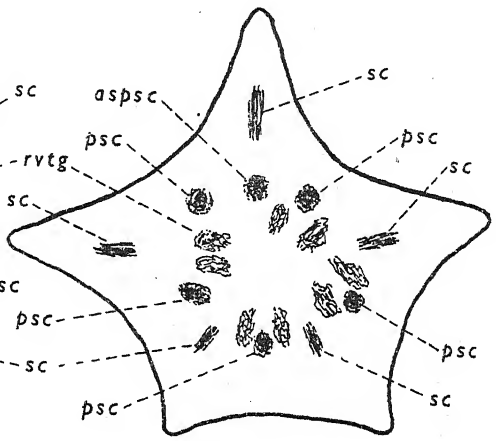


Fig. 9.

Fig. 8. *Primula acaulis*. Transverse section of a flower of the form K5 C7 (through duplication of two petals) A5 antesepalous staminal bundles (three in process of disappearing) +7 antepetalous stamens, taken at the level of origin of the perianth cords showing duplication of two petal-stamen cords.

Fig. 9. The same of a flower of the form K5 C6 (through the presence of an additional petal on a sepal radius) A5 antesepalous staminal bundles (the one in line with the sepal upon which the additional petal is superposed being represented only by the branch system present in the petal lobe on each side, the whole of the main bundle in the tube being absent) +6 antepetalous stamens.

(*a*) Morphological variations resulting from a like change of ground-plan on both sets of radii so that the several whorls, as in the type, are isomerous and the flower remains actinomorphic anatomically as well as morphologically.

Flowers with oligomerous or pleiomerous perianth whorls are of common occurrence in *Primula*. True tetramery was observed most frequently in primrose, six-, seven- and eight-merous flowers in polyanthus. Indeed in some polyanthus plants almost as many flowers on the earlier trusses were hexamerous as pentamerous, all the earlier flowers on each truss being six-merous. Hexamery is also fairly common in *P. malacoides* and among species of other genera in *Androsace* (e.g. *A. primuloides*) while six-, seven- and eight-merous flowers were once observed among the few flowers in the lowest whorl of an inflorescence of *Hottonia palustris*. It must be emphasized that the calyx and corolla of the tetramerous and of the

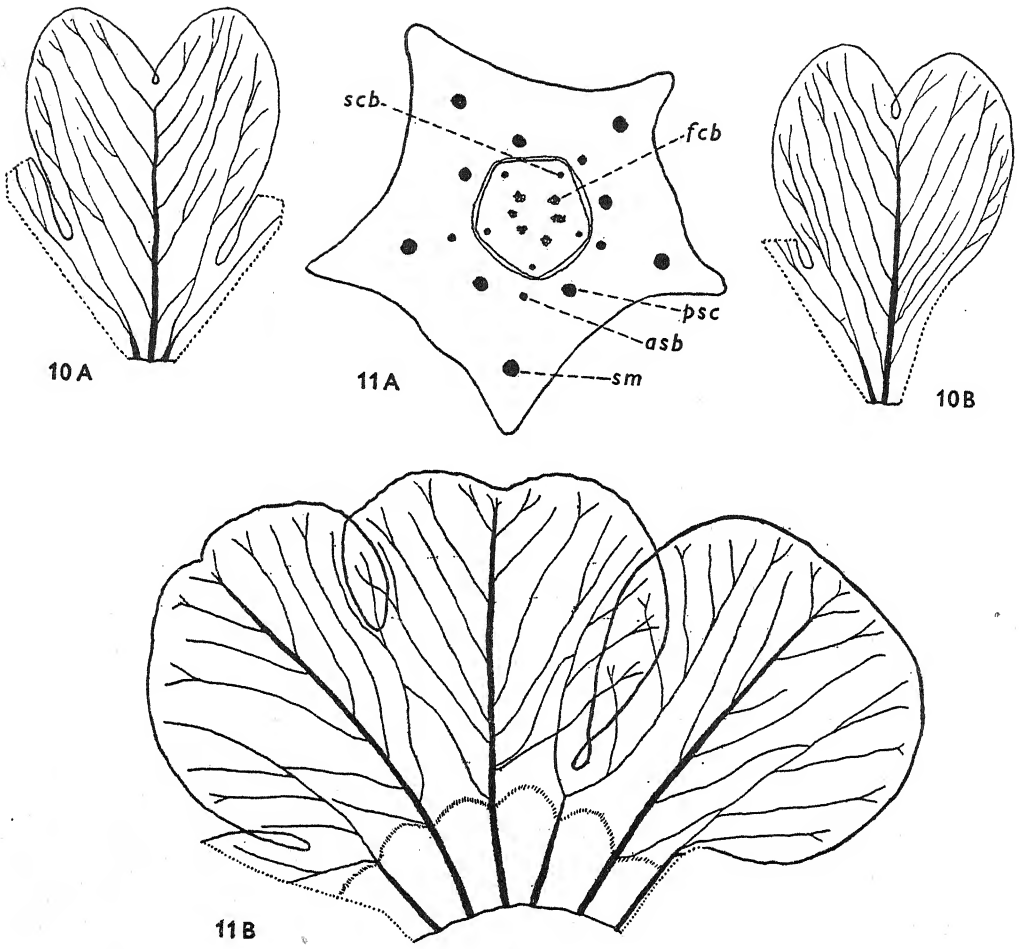


Fig. 10. *Primula acaulis*. Two petals of a flower, the one showing the petal midrib system together with an antesepalous staminal marginal system on both sides (A), the other having the latter system on one side only (B). In A anastomosis between the systems occurs at one point on each side.

Fig. 11. Polyanthus. From a flower of the form K 5 C 5, two being partially fused A 4 antesepalous bundles + 5 antepetalous stamens G 5 antesepalous sterile carpels + 6 fertile carpels. An antesepalous staminal bundle is wanting between the two imperfectly segmented petals. A. Transverse section (semi-diagrammatic). B. One normally segmented (right) and two imperfectly segmented petals. In the former an antesepalous staminal marginal vascular system on both sides, in the latter on one side only. Anastomoses between the staminal and petal midrib systems occur at one or two points.

hexamerous flower constitute single whorls, not two di- and trimerous sub-whorls respectively. This is shown both by the aestivation and by the sequence in which the midrib bundles originate. Even in the more symmetrical tetramerous corolla the scheme of overlap is variable; in the hexamerous form it is still more so. In most tetramerous corollas one petal overlaps, and the opposite petal is overlapped by both its neighbours, the other two petals having one edge outside and one inside their neighbours. Less often the arrangement is convolute. Only very rarely do two opposite petals lie outside the other two. Among many hexamerous corollas no single example was found in which three petals lay wholly outside the alternate

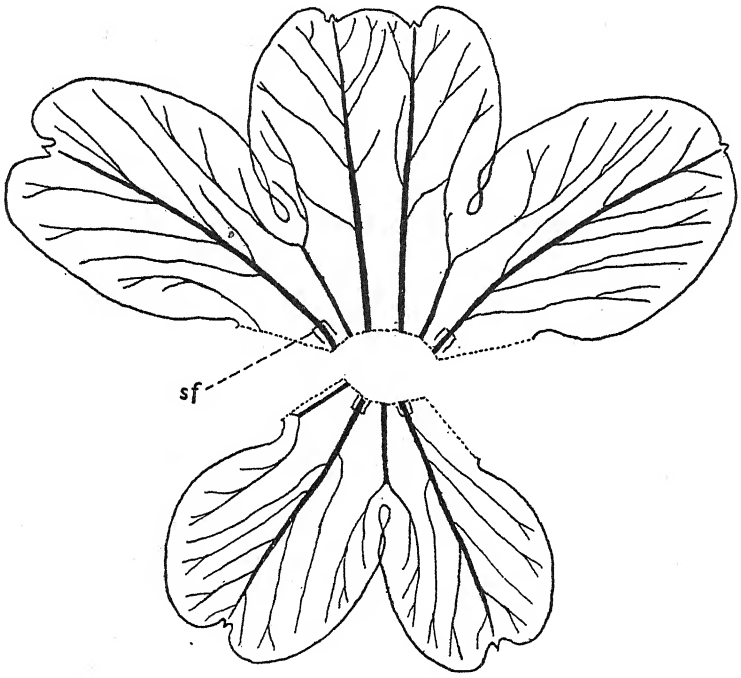


Fig. 12. Polyanthus. Flower with the corolla and androecium of the form C6 forming only five segments through complete fusion of two petals A3 antesepalous staminal bundles + 4 antepetalous stamens. An antesepalous staminal bundle is wanting between the two fused petals which are highly exceptional in that they carry no stamens.

three. In some flowers two petals are external to the others, in others one or, if the arrangement is convolute throughout, none. Furthermore, the trunk cords which furnish the sepal midrib bundles do not turn out from the central cylinder in two successive sets of three, but those on the side on which the sepals are more widely spaced emerge earlier than those on the opposite side and the trunk cords on the petal radii arise in similar sequence.

In these several isomerous forms the anatomical relations are otherwise similar to those described above for the pentamerous type and these forms need not therefore concern us further.

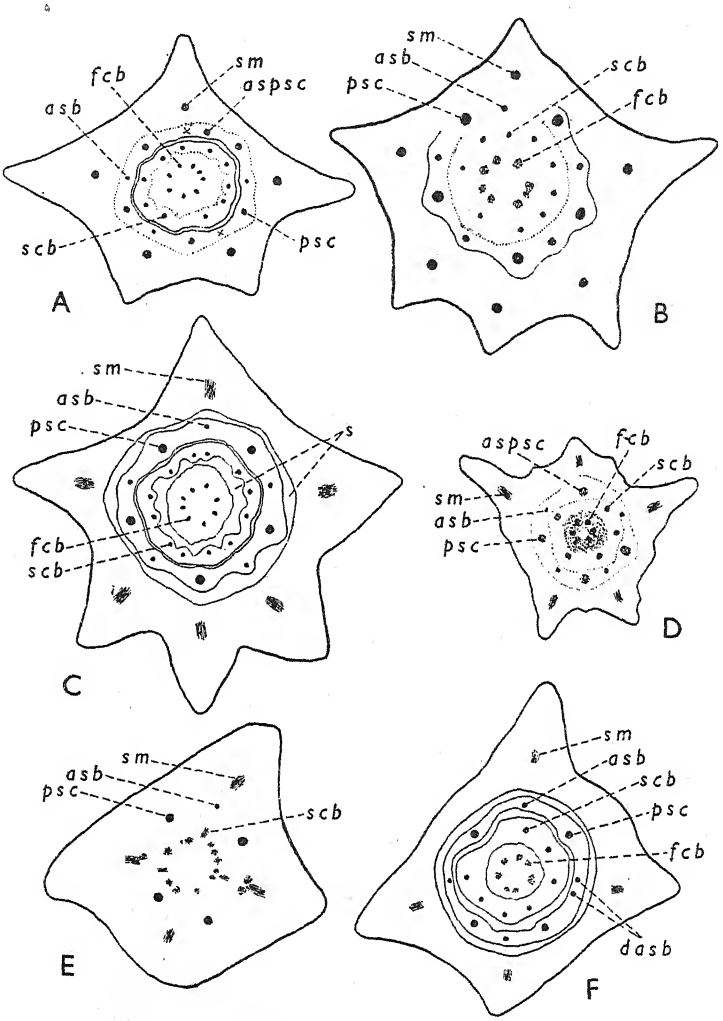
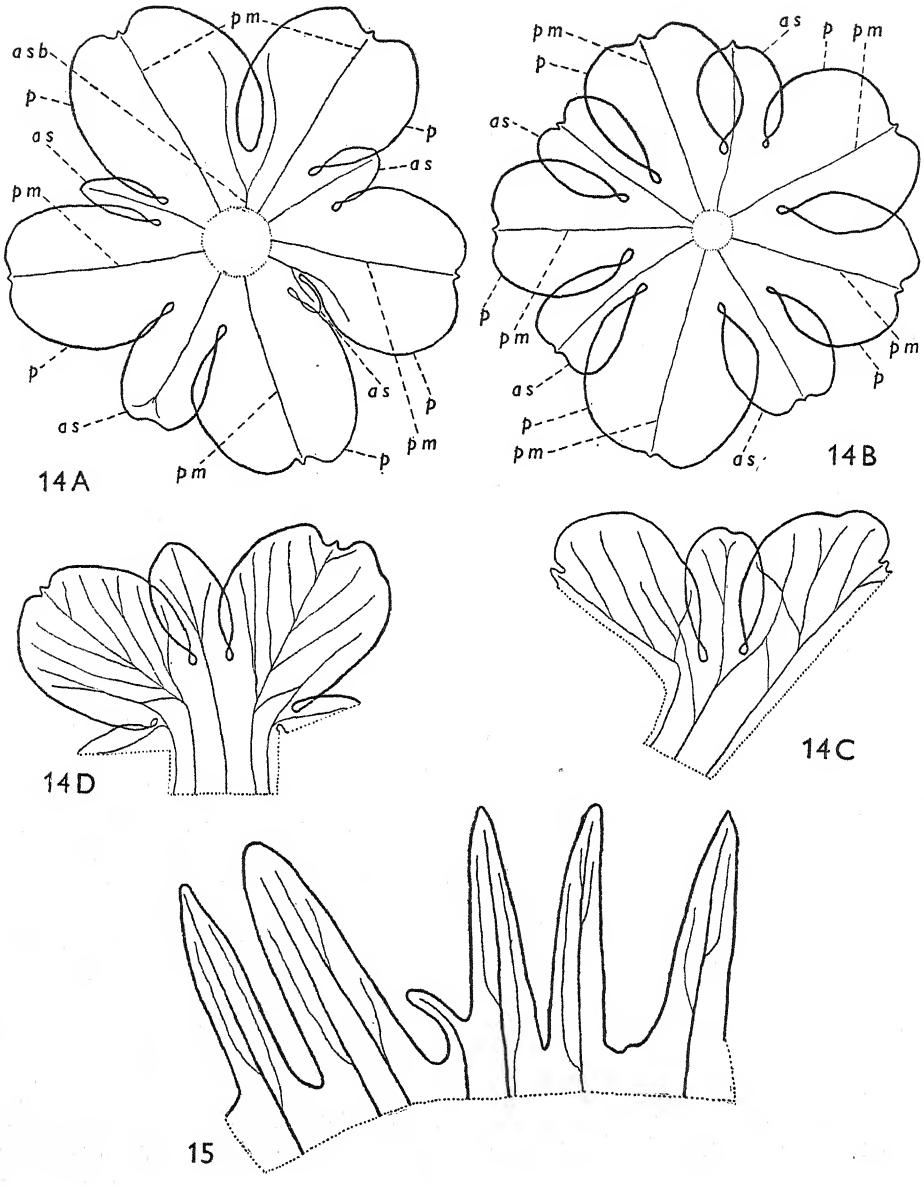


Fig. 13. Polyanthus. Transverse sections of exceptional flowers (semi-diagrammatic). A. From a flower of the form K5 C6 A3 full-length and 2 short-length (indicated by ×) antesepalous staminal bundles+6 antepetalous stamens G10 sterile+8 fertile carpels. The cord for the additional petal and stamen originated in line with, or immediately beside a sepal cord (centre back) but later turned slightly in its course towards the adjacent petal cord on one side. This deviation from the sepal radius permitted the development of a short-length antesepalous staminal bundle on this radius. Since no construction proper to a sepal radius was developed on the radius between the two approximated petal cords these petals were furnished with a separate marginal vascular system on one side only. B. From a flower of the form K6 C5 A5 antesepalous staminal bundles+5 antepetalous stamens G9 sterile+7 fertile carpels. Owing to the approximation of the additional sepal (centre front) to its neighbour on one side (left front) no construction proper to a petal radius takes place between them; furthermore, no antesepalous staminal bundle is formed on the radius of the additional sepal. This ground-plan furnishes each petal with a separate marginal vascular system on both sides. C. From a flower of the form K6 C5 A5 antesepalous staminal bundles+5 antepetalous stamens G12 sterile +8 fertile. Owing to the close approximation of three sepals through the formation of an additional member (centre front) there is no construction proper to a petal radius on the two intervening radii. This lack is balanced by the development of a petal cord on the radius of the additional sepal in place of an antesepalous staminal bundle. Each petal as in B has a separate marginal vascular system on both sides. D. From a flower of the form K5 C4 A4 antesepalous staminal bundles+4 antepetalous stamens G5 sterile+6 fertile. As in C the lack of petal cords on the two radii between three neighbouring sepals is balanced by the development of a petal cord on the radius of the middle sepal of this trio (centre back) in place of an antesepalous staminal bundle and each petal has a separate marginal vascular system on both sides. E, F. From a flower of the form K4 (unequal) C4 A5 antesepalous staminal bundles through duplication at the level of origin of one bundle (right)+4 antepetalous stamens G7 sterile+7 fertile carpels.

(*b*) Morphological variations not associated with a change of ground-plan and hence not arising from a corresponding change affecting both sets of radii.

Variations coming under this head are brought about in various ways, through duplication, through fusion partial or complete (i.e. through partial or complete failure in normal segmentation), through suppression and more rarely, through substitution; or through a combination of these causes.

When duplication occurs it may come about through bipartition of the axis and hence of the central vascular cylinder and may then take place either beneath or above the exertion level of the perianth. Or it may occur only in individual sectors outside the central cylinder so that only individual floral members and the corresponding midrib bundles become doubled. When bipartition occurs at the top of the pedicel, but below the exsertion level of the perianth, twin whole flowers are formed. In such an instance observed in *P. malacoides*, both flowers were hexamerous but otherwise typical. In another example from the same species where bifurcation had taken place above the exsertion level of the perianth, two separate ovaries had developed within a common perianth of 9 sepals and 9 petals. Such constructions are rare, but duplication of individual perianth members is exceedingly common. Such doubling may occur at any level. Two trunk cords may issue from the central axial cylinder side by side (Fig. 8); or the single trunk cord proper to a perianth radius may fork at some point in its course after leaving the central cylinder (Figs. 7, 16, 18 B); or the normally single androecium bundle detached from a perianth cord may originate as two separate strands (Fig. 13 E). In these circumstances twin members replace the normal single member, each with a midrib and each in the case of petals, with rare exceptions, carries a stamen. Such twin perianth members may show the normal degree of segmentation or they may be more or less completely fused (Figs. 11 B, 12, 16 B, 17 B, 18 A). If normal segmentation occurs, single-whorl pleiomery without increase in the number of primary construction radii and zygomorphy result. If, on the other hand, there is complete fusion, it is obvious that although the two perianth whorls show the same number of segments they are no longer isomerous. Examples of sectorial duplication were frequently observed in flowers with the construction K5 C6 and K5 C7 owing to duplication of one and of two petals, respectively. Independent pleiomery in the calyx, as e.g. in a flower with K6 C5 is less common. When it occurs it is not always easy to determine whether the construction represents an increase in the number of primary construction radii in the calyx (primary pleiomery) or whether it results from duplication on one of the original construction radii (secondary pleiomery). When forking of a sepal cord occurs at some point after it has left the central cylinder it is then clear that the pleiomery is of the latter type (Fig. 16 A). But if two sepal cords emerge from the cylinder separately, but in close proximity, it may appear uncertain whether this arrangement indicates extreme unequel spacing of x sepals or a precocious forking of the vascular unit proper to a single sepal in an x-1-merous calyx (Fig. 17 A). It must be made clear that the absence of a petal cord between the two approximated sepal cords does not serve to distinguish between these alternatives. For though such an intervening petal cord is invariably absent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Figs. 14, 15.

between the two forks of a duplicated sepal cord, it may also be missing when two sepal cords which undoubtedly belong to separate radii are insufficiently widely spaced; in other words, when the intervening sector not involved in, nor affected by, the sepal development on each side is too narrow at the critical level to allow of the physiological condition preceding morphological development reaching the grade required in order that such development should follow (see Fig. 5).

(2) The gynaeceum and fruit

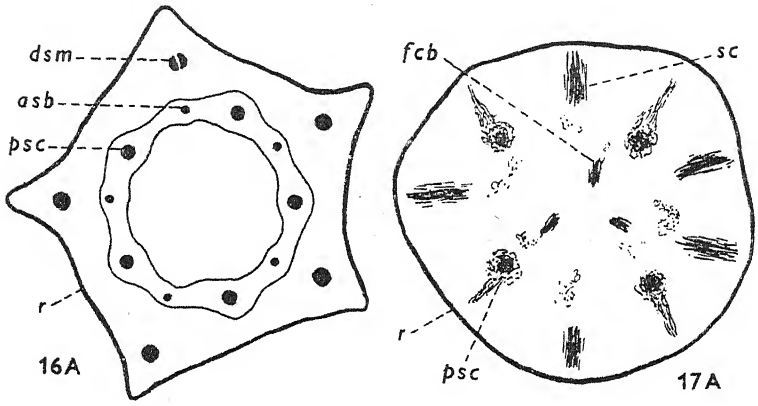
Evidence has already been given in earlier accounts showing (1) that the gynaeceum is constructed of two carpel whorls, the outer forming the ovary wall, style and stigma, the inner together with the non-vascular prolongation of the axis constituting the central ovuliferous column; and (2) that the full gynaeceal ground-plan in types with pentamerous outer whorls is G 10 + 10, but that this ground-plan is not often realized in its entirety (Saunders, 1932, pp. 265–280 and 286, 287). It is now possible to add further details concerning the carpel relations.

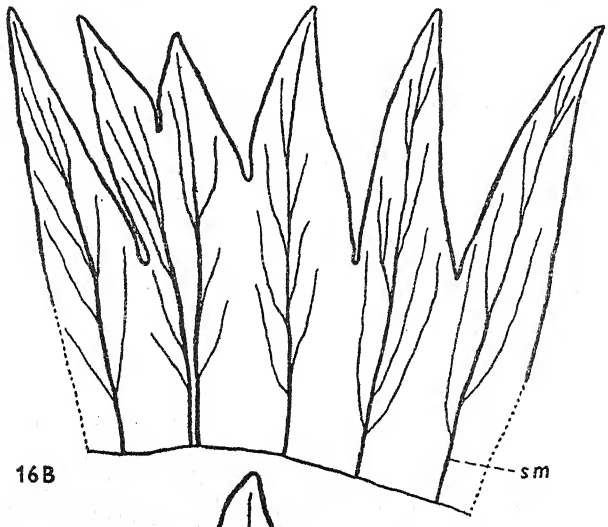
Reconstruction of the residual vascular cylinder takes place after the emergence of the perianth-androecium bundles. It thus becomes possible for varying numbers of carpel bundles to be organized in both whorls, as may occur even in flowers from the same individual. It results that there is no constant relation between the numbers of carpels in the two whorls nor between the number of outer carpels and of the members of the perianth-androecium whorls. During this process of re-construction the vascular elements for the inner carpel bundles usually become defined before those for the outer whorl.

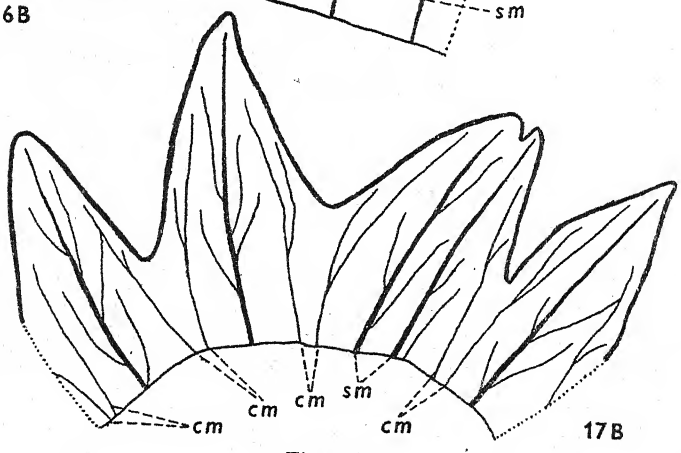
Legends of Figs. 14, 15

Fig. 14. *Primula floribunda.* A, B. The border of the corolla-androecium tube from two flowers in which four members of the antesepalous staminal whorl have attained separate morphological form as petaloid lobes. Petal and staminode main vascular bundles are represented but with three exceptions (in A) all lateral veins have, for the sake of simplicity, been omitted. In A the staminodes vary in size. In the two of medium size the midrib has out-topped the laterals and the apex is convex. In the largest the last laterals have out-topped the midrib and the apex is retuse. In the smallest the relation of midrib and outline corresponds with that of the two medium-sized members, but a basal lateral on one side provides a marginal system to the adjacent petal on that side. The vascular system of the missing fifth antesepalous staminal member is similar to that of the normal type, the midrib coming to an end at the level of the fork which furnishes a marginal system to the petal on either side. In B the staminodes are all similar and larger. In outline they resemble the petals and thus produce an illusory appearance of a double corolla. The whole of the vascular system of the missing fifth antesepalous staminal member has disappeared, so that only nine bundles turn outwards from the centre as compared with ten in A. C, D. Portions of the border of the corolla-androecium tube from two other flowers with a single staminode together with the entire corresponding vascular systems. C. The staminode together with half the neighbouring petal on each side. Staminode apex retuse in accord with the terminal branching of the midrib bundle. The petal vascular systems proper anastomose at one or two points with the marginal systems derived from the two basal branches of the staminode main bundle. The staminode system is exceptional in that these two basal branches originate at very different levels. D. The staminode together with one whole petal and a portion of another on each side. Staminode apex convex in accord with the prolongation of the main bundle above the highest laterals. Both petal systems proper anastomose at one point with the marginal system derived from the neighbouring staminode bundle.

Fig. 15. *Primula sertulum.* The calyx from a K7 C7 perianth split lengthwise and opened out. Only five of the sepals attain separate form, one of them being very small. Two others (2nd and 3rd from left) are completely fused.







Figs. 16, 17

When ten sterile carpels are present the corresponding vascular bundles usually lie on the sepal and petal radii, those on the one set of radii being often more strongly developed than those on the other set. When only five such carpels are present and equally spaced their position varies in accordance with the manner in which the corresponding midrib bundles are developed. If these bundles turn outwards from the central cylinder, they do so, in accord with the law of alternation, on the sepal radii; if they are organized centripetally, they lie on the petal radii. When intermediate or higher numbers (6–9 or > 10) occur, the bundles are usually unequally developed and unequally spaced. The radial position of the bundles of the inner (fertile) carpel whorl, owing to the small diameter of the cylinder, is not easily determined with certainty, but unequal spacing, as in the outer whorl, is often well marked.

Now and again flowers of cowslip and primrose occur with a deformed or monstrous gynaeceum. These flowers may be otherwise almost normal morphologically or they may also show considerable modifications in the outer whorls. Examples of abnormal gynaecea have been described and figured by Masters (1878), but his account deals solely with outward form. The clue to the interpretation of these forms which is furnished by the anatomical evidence is lacking. It chanced that flowers came under my notice from two plants, a cowslip and a primrose, showing various malformations in the gynaeceum. The cowslip produced some virescent flowers in 1933. Cross-sections of these flowers revealed a normal morphological construction in the outer whorls (Fig. 21 A). But the midrib bundles of the outer (sterile) carpels were all unusually strongly developed. As the ovary enlarged, the wall formed of the sterile carpels became fitted into the surrounding ring of perianth-stamen tissue as into a mould. But as the diameter of this mould soon became insufficient to accommodate the enlarging ovary, the wall was thrown into sinuous folds and assumed a lobed contour (Figs. 21 B–D). The separation of the central column of fertile carpels from the ovary wall was gradual and irregular and

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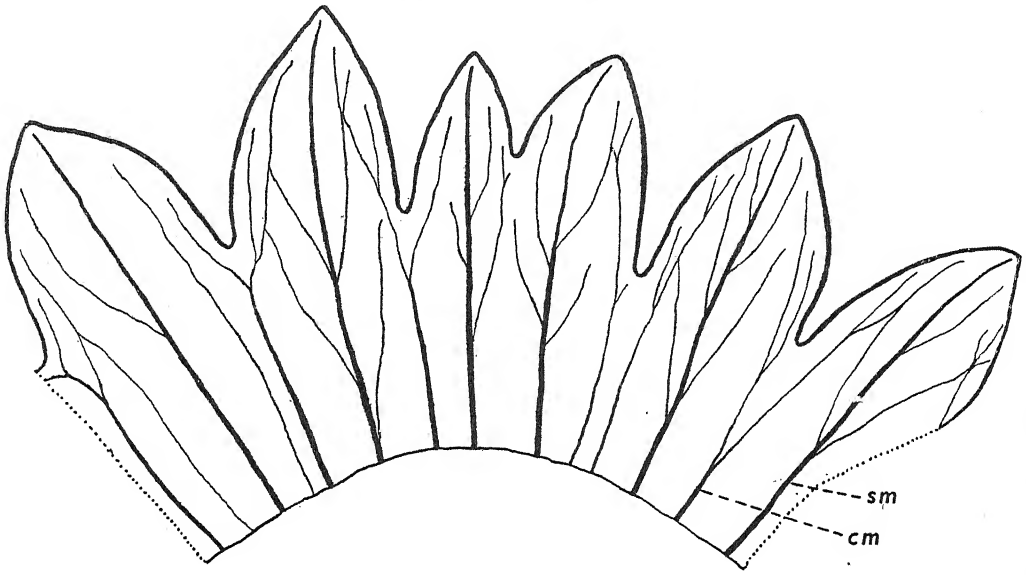
Legends of Figs. 16, 17

Fig. 16. Polyanthus. From a flower of the form K6 (through duplication of one sepal C5 A5 antesepalous staminal bundles + 5 antepetalous stamens. A. Transverse section of the calyx and corolla-androecium ring immediately above the level at which they become free from the ovary (not represented). The duplicated sepal cord is seen above. Since the antesepalous staminal bundles are detached from the sepal cords below the level at which duplication occurs, only one such bundle is present on the radius of the duplicated cord as on those of the single cords (semi-diagrammatic). B. The exserted calyx split lengthwise on the radius indicated in A by *r* and opened out. The two sepals resulting from duplication (2nd and 3rd from left) are incompletely segmented.

Fig. 17. *Primula Forbesii.* From a flower of the form K5, two being almost completely fused C4 A4 antesepalous staminal bundles + 4 antepetalous stamens G5 sterile + 3 fertile carpels. A. Transverse section of the flower base. The five cords which furnish the five sepal midribs have turned out from the central cylinder, the two on the right arising nearer together than the others. The four petal-stamen cords are in process of turning outwards. There is no corresponding cord between the two approximated sepal cords. In the centre the bundles of the three fertile carpels. The residual vascular elements left behind by the outgoing perianth cords have not yet become organized into the five sterile carpel midribs. B. The exserted calyx treated as in Fig. 16 B, the line of section being indicated by *r* in A. Two sepals (2nd and 3rd from right are almost completely fused). Between the midrib bundles the four pairs of commissural marginal bundles detached at a lower level from the petal-stamen cords. The two fused sepals have a commissural marginal system on one side only.



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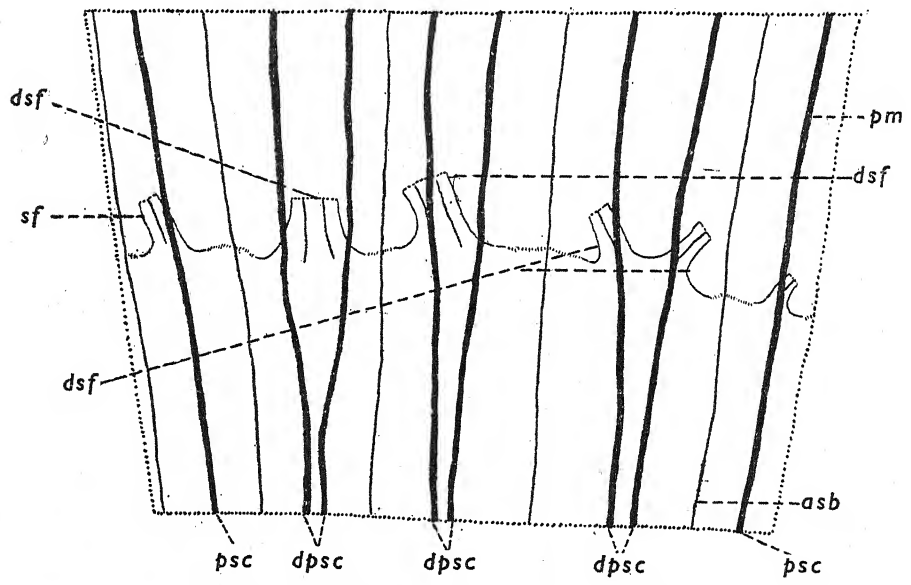

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Fig. 18. *Primula malacoides*. From a flower of the form K6 (through duplication of one sepal) C8 (through duplication of three petals) A5 antesepalous staminal bundles + 8 antepetalous stamens (the filaments of the pair borne by two of the duplicated petals being fused together but having separate vascular bundles, those of the pair borne by the third duplicated petal being separate). A. The calyx split lengthwise and opened out. A commissural marginal vein is present only on one side of each of the two sepals resulting from duplication since no antepetalous cord from which these veins are normally derived intervenes between two sepals arising by duplication. B. Part of the corolla-androecium tube from the same flower split lengthwise and opened out.

led in the first instance to the appearance of small separate lacunae corresponding with the number of lobes in the wall, thus producing an illusory effect at this level as of a multilocular ovary. In this specimen the sterile carpels remained coherent throughout, terminating above in the usual single style and capitate stigma. This plant which had been uprooted was replanted and flowered the following year (1934). Only a few flowers were produced and these were all normal, the one which was sectioned having the same number of carpels (9+5) as the 1933 flowers here figured.

In the second specimen, a primrose plant, four early flowers had a monstrous gynaeceum, the rest were normal. These four flowers, which I was able to examine in detail through the courtesy of Mr J. L. Crosby who had bred the plant, all showed

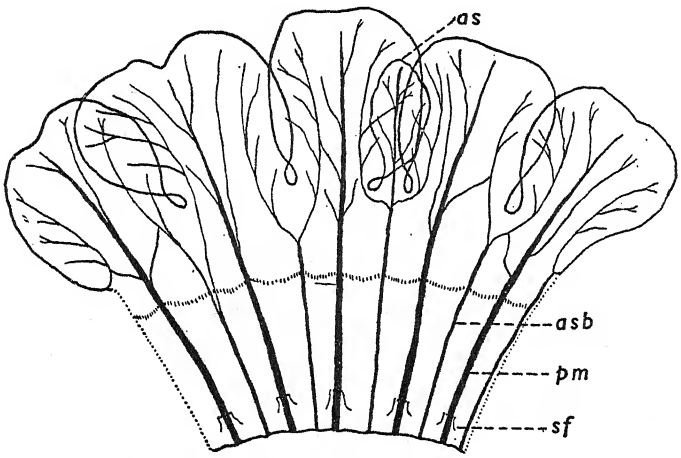

Fig. 19. *P. Bessiana.* Part of the corolla and androecium of a flower of normal construction except that a member of the antesepalous androecium has attained separate morphological form as a petaloid staminode. In accord with this development the corresponding antesepalous staminal bundle is prolonged above the level of origin of the pair of primary laterals and gives rise above to other laterals (monopodial branching). The other antesepalous staminal bundles show the normal dichasial branching.

some departures from type in the outer whorls (Fig. 22). As in the cowslip individual described above, the ovary had a lobed contour and the sterile carpel midrib bundles were exceptionally strongly developed and were unequally spaced so as to appear as four groups of three, three, two and two, respectively. But the malformation in the gynaeceum went much further than these features. Separation of the corolla-androecium tube from the ovary wall began in such a way as to leave connecting strips of parenchyma on the radii between the groups of sterile carpels (Figs. 22 A, B). These strips became ruptured at a higher level (Figs. 22 C, D) leaving the inner portions incorporated in the ovary wall (Fig. 23). Towards the top of the ovary the wall had split longitudinally along these parenchymatous tracts giving rise in this way in the specimen figured to four styles corresponding with the four groups of sterile carpels, each terminating in a stigma. In other flowers on this plant the break-up into the component carpels was more complete, some styles being

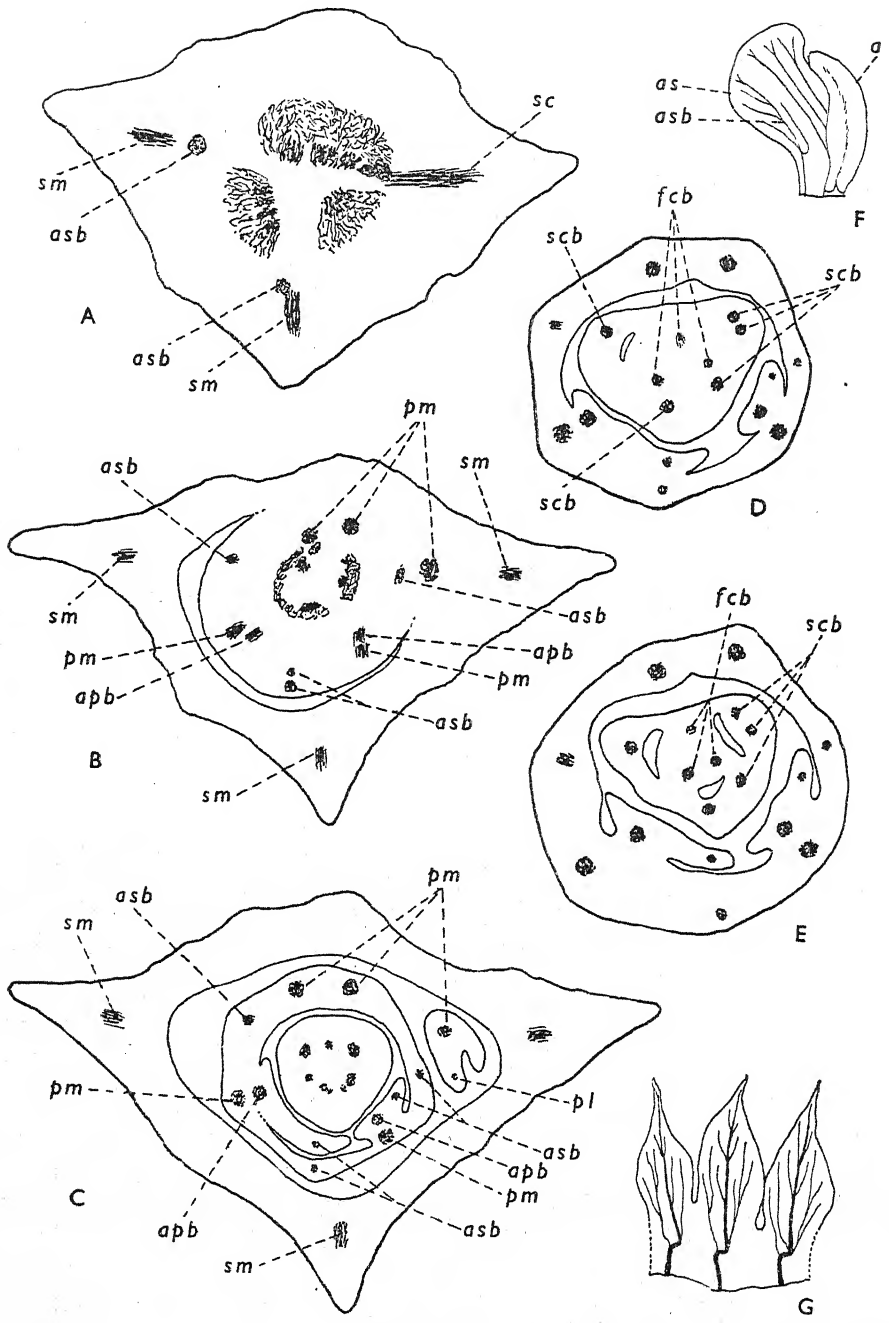
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Fig. 20.

ormed of individual carpels. Some, unable through crowding to elongate upwards, were bent down in the form of an inverted V (thus ∧) the terminal stigmatic area being directed downwards and ending in the ovary cavity.

In most (? all) Primulaceae the cell walls in the upper hemisphere of the ovary become thickened. If the tension between the upper thick-walled half and the lower thin-walled half is greater than that between the sterile carpel midribs and the intervening non-vascular sectors, the ripe capsule splits transversely (pyxidium). If the tension is greater between the sterile carpel bundles and the intervening sectors, the capsule splits longitudinally in line with the bundles. If these bundles are equally developed, splitting takes place in line with all of them. If those on the one set of radii are more strongly developed than those on the other set, splitting may occur only in line with those more strongly developed. Hence the fruit valves may be of unequal width and if five and of similar size they are antesepalous in some types and antepetalous in others.

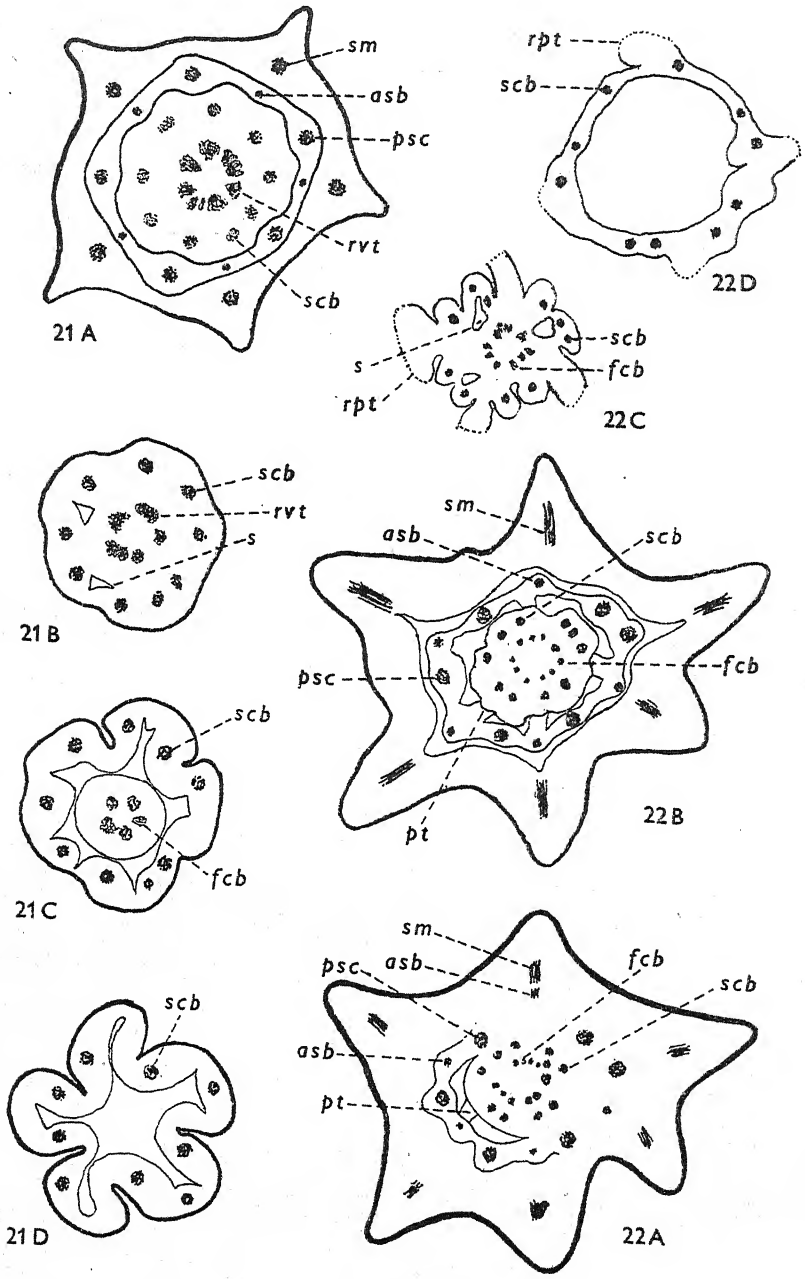
Summary and conclusions

1. Numerous morphological departures from type occurring sporadically in flowers of *Primula* together with the anatomical adjustments accompanying them are described.

2. The calyx and corolla always consist of single whorls whatever the number of sepals and petals present (4–9), but a petal is sometimes developed on a sepal radius, the corresponding midrib-bundle replacing wholly or in part the vascular system of an antesepalous member of the androecium normally present on each sepal radius.

Legend of Fig. 20

Fig. 20. Polyanthus. Transverse sections taken at successively higher levels from an exceptional flower of the form K 3 C 5 A 3 antesepalous staminal bundles + 2 antepetalous stamens G (malformed) 5 sterile + 3 fertile carpels. A. At the level at which the three cords on the sepal radii turn out from the central vascular cylinder. One (left) has already broken up into sepal midrib and antesepalous stamen bundle. One (front) is about to become resolved into these components; the third (right) is not yet free from the cylinder. B. After partial separation of the calyx from the corolla-androecium tube and the organization of the vascular bundles of the corolla and androecium whorls, one petal bundle (right) being superposed upon a sepal and two (back) arising on the radius between the two widely spaced sepals. One antesepalous staminal bundle seen in A (front) has branched, the inner strand is the continuation of the main (midrib) bundle, the outer one represents the primary laterals at this level conjoined. The third antesepalous staminal bundle (belated owing to the exceptional formation of a petal midrib on or beside this radius) is seen on the right. The cords of the two petals which carry fertile stamens (right and left front) have given rise to the petal midribs and stamen bundles. In the centre the residual vascular elements for the gynaeceum. C. After complete separation of calyx, corolla-androecium tube and ovary. The petal superposed on a sepal (right) has become disjoined singly. The antesepalous staminal bundle on the same radius has branched in the same manner as the corresponding front bundle (see B). The branch derived from each of these two antesepalous staminal bundles runs up the corolla tube, the prolonged main bundle passes into a petaloid structure which becomes free from the tube and is fused laterally with the adjacent fertile stamen. These petaloid structures represent antesepalous staminodes. In the centre the ovary with a ring of vascular bundles not yet organized into individual carpel bundles. D. The corolla-androecium tube and ovary. The carpel bundles (five sterile three fertile) are now organized. The fertile carpel column has become disjoined from the ovary wall at one point (above left). E. The same after the fertile carpel column has become disjoined from the ovary wall at two additional points. F. The anther of a fertile stamen with a dorsal petaloid expansion fused with the adjacent antesepalous petaloid staminode. The vascular bundle of the fertile stamen has given rise to a branch system in the petaloid expansion. To the left the separate pinnate system of the staminode. G. The calyx split lengthwise and opened out (less highly magnified than Figs. A–E).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Figs. 21, 22.

3. Complete reconstruction of the residual cylinder takes place between the organization of the perianth-androecium vascular systems and of those for the gynaeceum, hence anatomical and morphological variations in the carpel whorls occur independently of those in the outer whorls.

4. Morphological variations in the perianth and androecium fall naturally into two categories, viz. those associated with a fundamental change of ground-plan and those superimposed upon the normal ground-plan.

5. When variations in these outer whorls result from a fundamental change of ground-plan involving both sets of radii, as in simultaneous oligomery or pleiomery of calyx, corolla and androecium, the flower remains isomerous and actinomorphic both morphologically and anatomically and the anatomical relations are similar to those in the pentamerous type.

6. When oligomery or pleiomery occurs in one or other perianth whorl, but not simultaneously and similarly in both whorls, the heteromerous flower becomes zygomorphic or asymmetrical both morphologically and anatomically, unless through lateral morphological fusion the outward form simulates an isomerous actinomorphic construction which anatomical evidence shows to be illusory. Since such lateral fusion is fairly common among sepals, petals and stamens, it follows that the vascular ground-plan is a more reliable guide to the number of floral members present than the scheme of segmentation, or in other words than the morphological ground-plan.

7. The incidence of isomerous pleiomery suggests that this condition may be to some extent dependent upon the inherited constitution of the individual plant exhibiting it; heteromerous pleiomery on the other hand appears rather to be the outcome of accidental spatial conditions existing at the appropriate moment in individual flowers.

8. In the pleiomerous calyx the sepals are sometimes equally, sometimes unequally spaced. When equally spaced the corolla is generally isomerous, when

Legends of Figs. 21, 22.

Figs. 21, 22. All from transverse sections taken, when in series, from successively higher levels

Fig. 21. *Primula veris*. From a virescent flower of the form K 5 C 5 A 5 antesepalous staminal bundles + 5 antepetalous stamens G 9 sterile + 5 fertile carpels. A. At the level at which the calyx, corolla-androecium tube and ovary are well-defined but not yet wholly disjoined. In the centre the residual vascular elements not yet organized into the fertile carpel bundles. B. The ovary at the level at which the sterile carpel whorl has begun to separate from the central column. C. The same after separation of the sterile from the fertile carpel whorl is complete. The lobing of the ovary wall and the unequal spacing of the sterile carpel midrib bundles are now more marked. In the central column the bundles of five fertile carpels. D. The ovary wall showing more pronounced lobing.

Fig. 22. *Primula acaulis*. From a flower of the form K 6 C 6 (through the development of a petal on the radius of a sepal and the absence of a petal between this sepal and its closely approximated neighbour) with petaloid venations of varying size A 5 antesepalous staminal bundles (through absence of a bundle on the radius of the sepal upon which a petal is superposed) + 6 antepetalous stamens G 10 sterile + 10 fertile carpels. A. The flower at the level at which the corolla-androecium tube has become disjoined on one side from the ovary except for a strip of specialized parenchyma which still connects the two structures on this side. The bundles for both sterile and fertile carpels are now defined but are not yet fully organized. B. The same after separation of the corolla-androecium tube from the calyx and from the ovary except for four unruptured strips of parenchyma. C. The ovary after rupture of the persistent strips of parenchyma seen in B. The ovary wall is becoming detached at points corresponding with the groups of irregularly spaced sterile carpels from the inner fertile carpels. D. The ovary wall.

unequally spaced the petal proper to the radius between the two nearest sepals may be absent.

9. The relations indicated in the preceding paragraph suggest that the development of a floral member affects the cells in an axial sector of a certain minimum width. If there is no unaffected sector between two neighbouring members of the calyx, or if such a sector is of insufficient width to permit development from a new focus, the petal proper to the intervening radius is not developed.

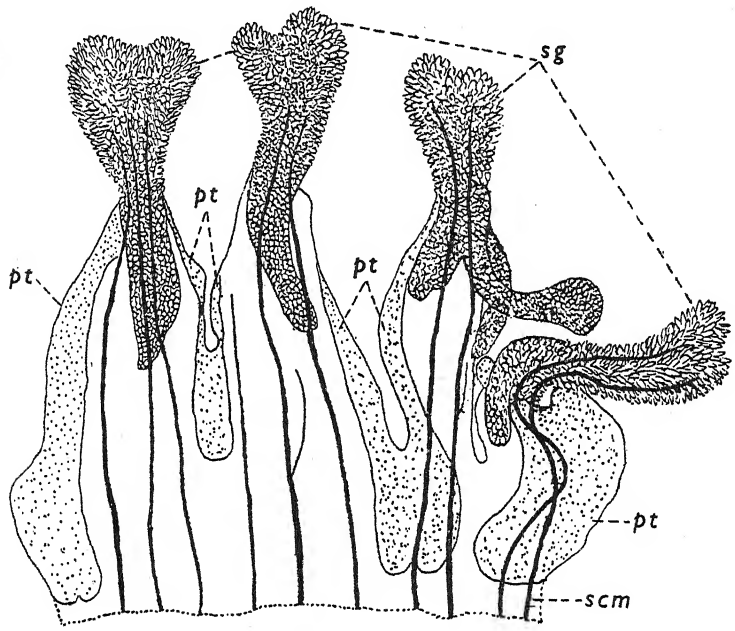

Fig. 23. *Primula acaulis*. Upper portion of the gynaeceum shown in Fig. 22 split lengthwise and opened out after being rendered semi-transparent, seen from the ventral face. Below, the upper part of the ovary wall of ten sterile carpels; above, four styles and four stigmas corresponding with the four groups (3, 3, 2, 2) of sterile carpels. One sterile carpel midrib ceases near the base of the corresponding style, the others are continued into the stigmas; two have formed a lateral branch. The tissue of the four stigmas is continued downwards into the ovarian cavity, ending in entire or separate tongue-like lobes. On the dorsal face between the groups of sterile carpel midribs prolongations of the specialized parenchymatous tracts which at a lower level connected the ovary wall with the corolla-androecium tube.

10. Independent pleiomery in the corolla may occur through duplication of one or more of the petals and of the corresponding cords or midrib bundles. Such duplication of the cords may take place as they emerge from the central cylinder or at any later point in their course up the corolla-androecium tube.

11. The number of antepetalous stamens, apart from some rare exceptions among flowers with a heteromerous perianth and duplicated petals, corresponds with that of the petals. But if when a petal is duplicated the two corresponding vascular cords lie near together in the corolla-androecium tube, the two corresponding stamens may be represented by a single double-sized filament or anther.

12. In the process of reconstruction of the residual vascular system after the organization of the perianth-androecium cords, the fertile carpel bundles are generally delimited before those of the sterile carpels.

13. The vascular bundles for the sterile carpels turn outwards from the central cylinder in some species; in others they are developed centripetally.

14. When more than five sterile carpels are present, the corresponding vascular bundles are usually unequally spaced and unequally developed.

15. If the sterile carpel bundles turn outwards from the central cylinder and are equally developed and spaced and are isomerous with the corolla-stamen cords, they turn out on the sepal radii. The ripe capsule then splits in line with the sepals and the fruit valves are antepetalous.

16. If the sterile carpel bundles are developed centripetally and are equally developed and spaced and are isomerous with the corolla-stamen cords, they usually lie on the petal radii. In such case, or if more than five bundles are formed, and if those on the petal radii are more strongly developed than the others, the ripe capsule splits in line with the petals and the fruit valves are antesepalous.

17. When the sterile carpels are equally developed, all the corresponding vascular bundles are prolonged up the style to the stigma; if they are unequally developed, the weaker bundles may come to an end at a lower level.

18. Abnormal ovaries occur occasionally (*P. acaulis*, *P. veris*) in which the ovary wall tears on the radii between groups of the sterile carpels or between an individual carpel and its neighbours. Each separate group or individual then generally bears a separate style and stigma.

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MECHANICAL STIMULATION AND RESPIRATION IN THE GREEN LEAF

PARTS IV AND V

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(With 7 figures in the text)

IV. THE EFFECTS OF STIMULATIONS AT SHORT TIME INTERVALS AND THE DEVELOPMENT OF FATIGUE

Introduction

In previous papers (1935, 1939, 1940) the author has shown that slight mechanical deformation of the cells of the lamina of starved foliage leaves greatly stimulates their oxidative respiration rate, and that a period of 2–3 days is required for their recovery from the stimulated condition. Successive stimulations described in these experiments were carried out after intervals of time sufficient for the normal starvation drift of respiration of the leaf sample to be reattained. From these experiments no measurements were obtained of the sensitivity of the leaf to stimulation during the recovery period. In order, therefore, to discover to what extent sensitivity persists during this period, and to investigate any possible fatigue effects of continued stimulation, experiments were carried out at relatively short time intervals.

Two distinct types of experiment were performed with detached leaves of cherry laurel at 22·5° C. In one the leaf sample was subjected to two successive stimulations only, the second taking place at varying intervals during the recovery period from the first stimulation. From the second effects, measures of the sensitivity of the leaf to stimulation during this period could be obtained. In the second type of experiment a series of consecutive stimulations at short time intervals was performed. In this way the development of fatigue phenomena was investigated.

Methods

The apparatus and methods used in the investigations were identical with those of the early experiments (1935, 1939). On the average, samples of from 15 to 20 leaves were used and stimulations, carried out by hand, were confined to the protoplasmic phase of the starvation respiration drift.

Results

(1) *Experiments with two successive stimulations at short time intervals*

In all cases it was found that the second stimulation of the pair produced a very definite and significant response, the respiration rising to a peak in the first or second reading, and then declining in a typical recovery curve. Results of three experiments with different time intervals between stimulations are drawn in Fig. 1. The first

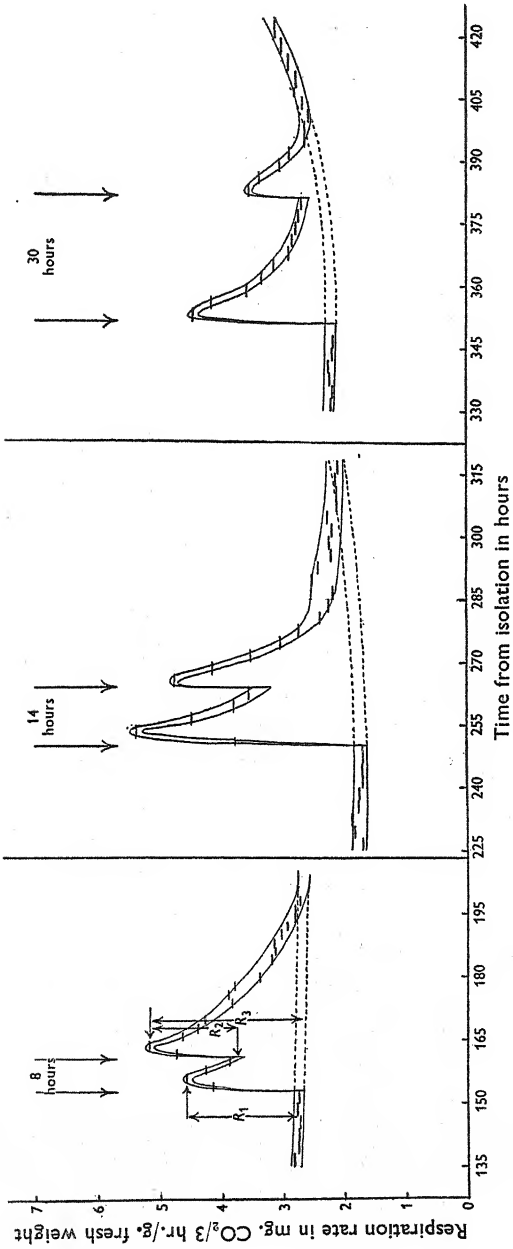

Fig. 1. Graphs showing the effects on the respiration of three samples of starved cherry laurel leaves of two successive stimulations at relatively short time intervals. The times of stimulation are marked by vertical arrows. The dotted lines are the interpolated normal respiration drifts.

important fact which emerges from these results is that the net rise of respiration after this second stimulation (R_2, reckoned from the actual respiration rate) is much smaller than the net rise (R_1) after the first stimulation. On the other hand, the peak value of the respiration rate after the second stimulation may in some cases surpass the peak value of the first stimulation effect. In this respect it has been found that a definite relationship exists between the relative heights of the two peaks and the time interval between the two stimulations. This relationship is illustrated in Fig. 2.

In this figure the ratios of the heights of the second peaks above the normal drift (R_3 in Fig. 1) to those of the first peak (R_1 in Fig. 1) are plotted against the time intervals between the two stimulations. There is a considerable amount of scatter of individual points, but the two smooth curves have been drawn at the limits of this scatter. The graph shows that, for short time intervals between stimulations, the second peak values are well above the first, i.e. the maximum augmentation of respiration is not attained by the first stimulation. Increase in the time interval results in a rapid fall in this second peak value, until, at 50 hr., it has been reduced to about 60% of the first. Still longer intervals show an eventual rise to equality with the first after about 90 hr. A typical single stimulation effect is drawn at *C*, showing that the time taken for the respiration to recover from a single stimulation corresponds roughly with the minimum point of the first curve.

Curve *A* reflects the variation in reaction of the leaf to a second stimulation during recovery from the first, and suggests the development of a depression in the reactivity which passes off only after an interval of about 90 hr. from stimulation. This effect is very reminiscent of a depressant effect on the respiration rate itself, which was noticed during the recovery from a single stimulation in a number of earlier experiments (1935). One such effect is drawn at *B* in Fig. 2. Although these last effects have been observed only during senescence, it is probable that they are present to a lesser degree in all the normal recovery curves, but are too small to carry the net respiration rate below the normal drift rate, and thus become obvious. It is probable that the effects on sensitivity and these latter effects on the respiration rate are closely connected and stress the complexity of the respiratory response to mechanical stimulation.

The form of curve *A* in Fig. 2 can be explained by postulating that the second stimulation effect is additive to the first, but that the sensitivity of the leaf to the second stimulation is lowered by the first stimulation. Subsequent recovery of normal sensitivity is much slower than the recovery of the respiration rate. This is illustrated by the theoretical curves in Fig. 3. Curve *A* is a recovery curve of respiration after the first stimulation. Curve *B* is a theoretical sensitivity curve, showing a reduction to 40% of the normal immediately after stimulation and a subsequent slow recovery after about 4 days. Curve *C*, deduced from curves *A* and *B*, is of heights of peaks of effects of second stimulations carried out at varying time intervals from the first. This curve has been constructed by multiplying the height of the peak of effect *A* by the value of the residual sensitivity (in percentage of normal) corresponding to the appropriate time interval, to get the theoretical second stimulation rise, R_2. This has been added to the appropriate values of the respiration

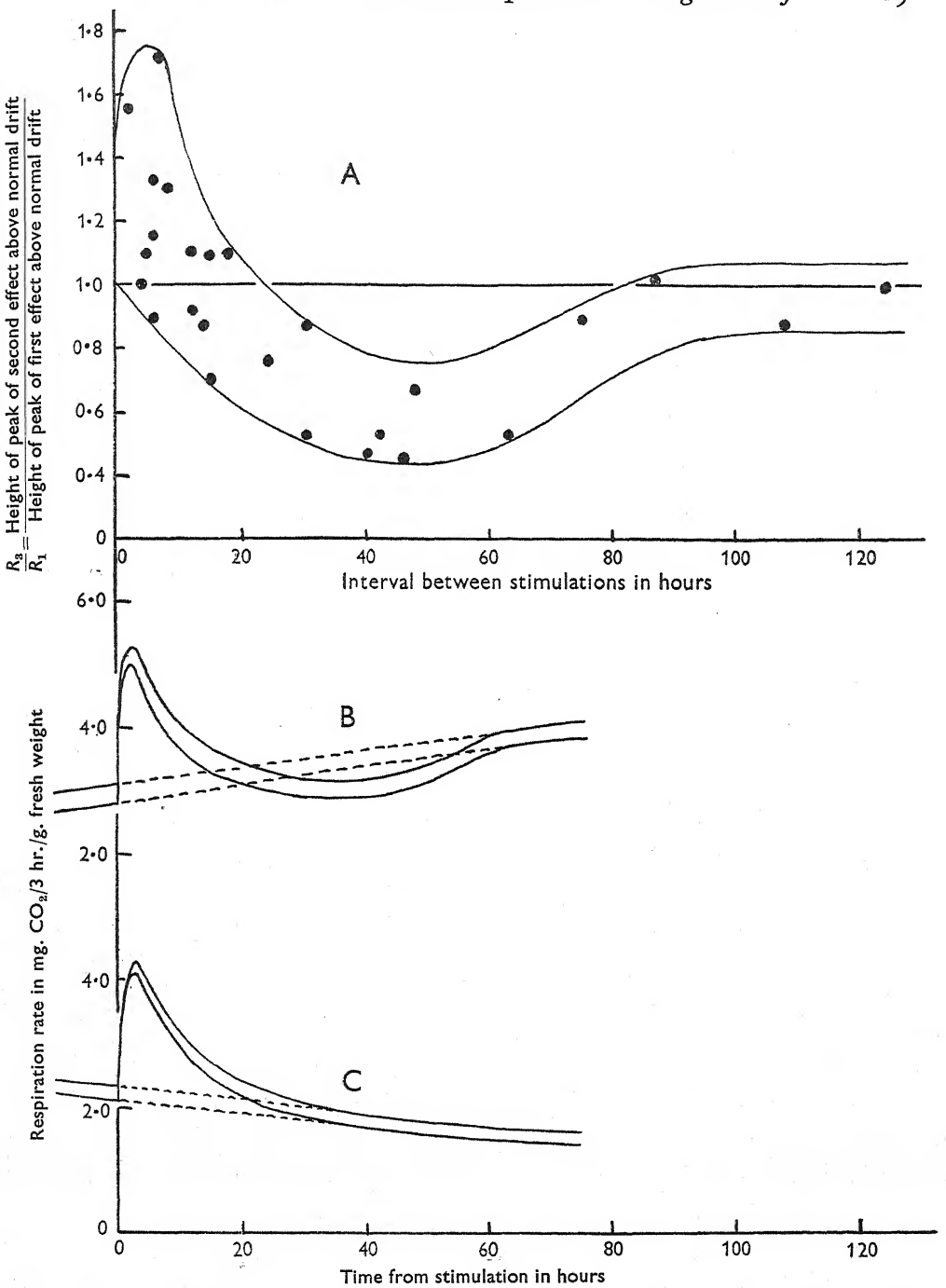

Fig. 2. A. Graph showing the relationship between heights of peaks of first and second effects, and the time interval between stimulations. B. Stimulation reaction showing secondary depressant effect. C. Normal stimulation effect.

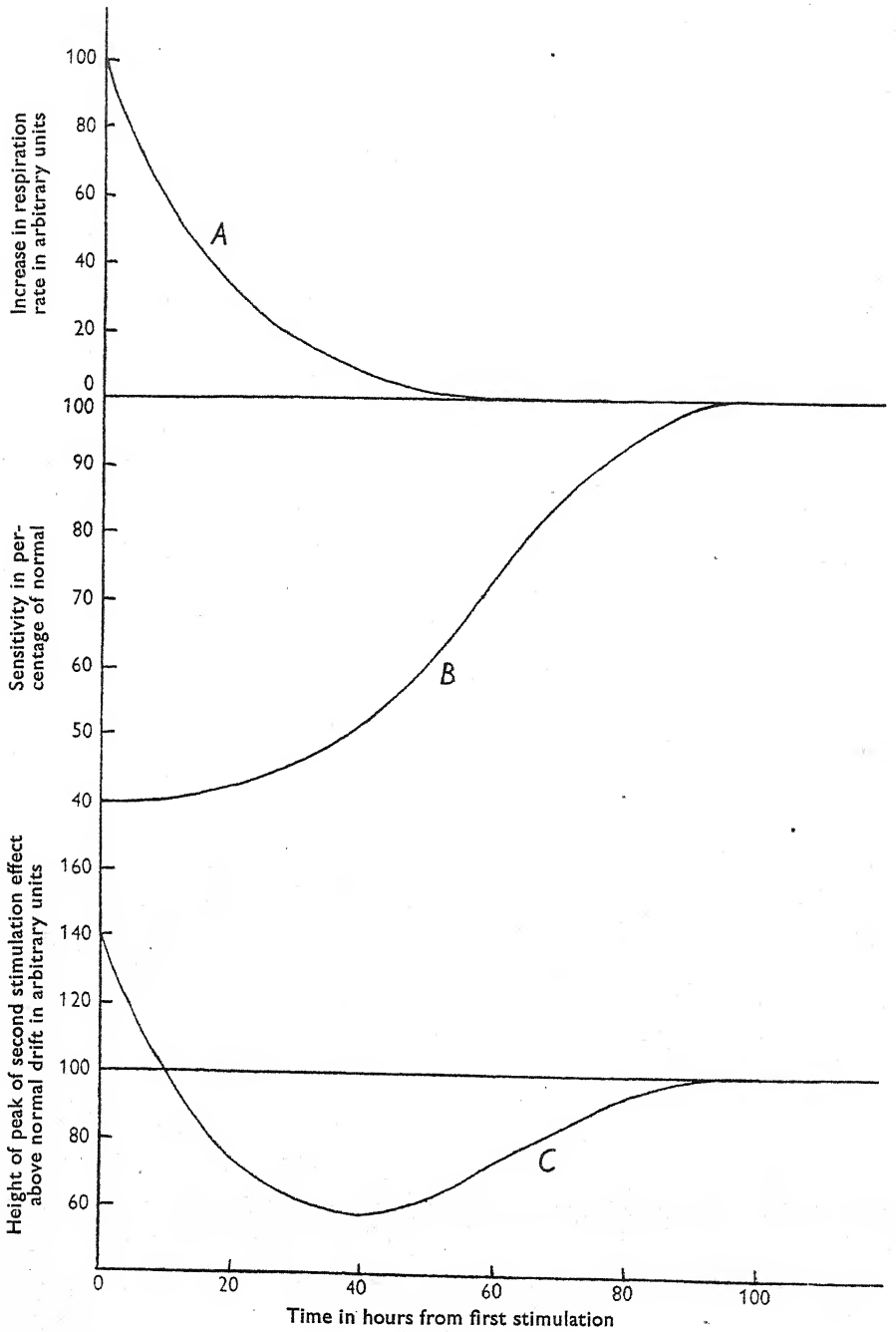

Fig. 3. Theoretical curves relating sensitivity of the leaf to stimulation (*B*) and relative heights of peaks of first and second stimulation effects (*C*) with the time interval between stimulations. Curve *A* is a normal respiration recovery curve from a single stimulation.

recovery curve *A* to obtain the heights of the peaks of the second effects shown in curve *C*. The configuration of this last curve corresponds very closely with the observed results of Fig. 2A.

(2) *Experiments with a number of successive stimulations at short time intervals*

The results of two parallel experiments are shown in Fig. 4, where the stimulation effects have been plotted as differences from the normal starvation drift of respiration. The times of stimulation are marked by the vertical arrows. Inspection of these curves shows that the same principles govern the reactions of the leaf to a number of stimulations at short time intervals as to a pair only of such stimulations. This suggests that such stimulations do not reduce the sensitivity of the leaf below

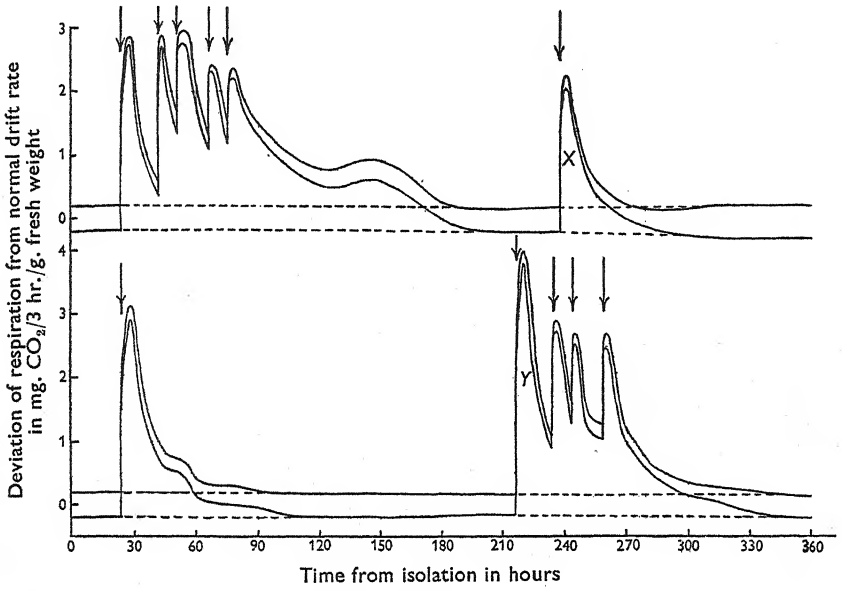

Fig. 4. Curves from two exactly similar leaf samples showing the effects of a number of stimulations at short time intervals. Results are plotted as differences between actual respiration rates and the normal starvation drift. Times of stimulation are marked by the vertical arrows.

that set by the first stimulation. A further point that accrues from the above curves is that this continued stimulation seems to have a slight depressing effect on the ultimate sensitivity of the leaf, as shown by a comparison of effects *X* and *Y*. Effect *X*, following a series of stimulations, is markedly smaller than effect *Y*, which follows only one previous stimulation. Two other similar experiments bear out this result.

It would be interesting to know the effects of continued stimulation, but such experiments are not possible with the present technique of stimulation by hand. It would be instructive to see whether a continuous high level of respiration, such as is suggested by these latter experimental results, could be maintained by these means over long periods of time.

V. THE RELATION OF MECHANICAL STIMULATION EFFECTS TO LEAF TURGIDITY

In 1935 Barker described an augmentation of the respiration of potato tubers resulting from gentle squeezing. He stated that the effect was closely correlated with the degree of turgidity of the tuber cells, being large when the tuber was wilted and zero when it was turgid. He deduced from this that the actual stimulus was deformation of the tuber cells.

The effects of cell turgidity on the stimulation effect in cherry laurel leaves have been investigated. Leaves were made to wilt while in the respiration chamber at 22·5° C. and then they were stimulated by hand as in previous experiments. The effects of this treatment on the respiration were closely followed.

EXPERIMENTAL

Preliminary experiments were carried out by passing $CaCl_2$-dry air over the leaves at 2 l./hr. instead of the customary moist air and at the same time removing the supply of water to the cut ends of the petioles. Stimulations were carried out at approximately five-day intervals during starvation as wilting proceeded. Under these conditions wilting of the leaves was slow and at the same time the onset of yellowing in the leaves was accelerated, so that the effects of wilting were complicated by senescence.

The results of such an experiment are shown in Fig. 5 with those of an unwilted sample for comparison. These curves clearly show the increase in the respiration rate on wilting, due presumably to an increase in the concentration of respirable substrates. This increase is unfortunately complicated by senescence, the accelerated onset of which is clearly apparent from comparison of the yellowing curves. The first stimulation carried out was just previous to the commencement of wilting and the resulting effect can be taken as a standard for the normal leaf sample. Subsequent effects during wilting do not show violent departures from this standard, but decrease with starvation in a way similar to those of the unwilted sample. These results suggest that wilting had no effect on the magnitude of the stimulation reaction.

These results were not regarded as satisfactory, however, owing to the onset of senescence during the slow wilting. Attempts were therefore made in succeeding experiments to induce rapid wilting in the leaf sample. To accelerate water loss the respiration chamber was fitted with four outlet and inlet tubes, one pair for the normal air current of 2 l./hr. passing through the pettenkofer tubes and the other pair for circulation of the air in the chamber at a rate of approximately 30 l./hr. through a closed drying circuit. The latter circulation was carried out by a small power-driven pump, the air being dried by fine-grain $CaCl_2$ in an absorption tube 3 ft. long and 1 in. in internal diameter. The air flowing into the system was also dried over $CaCl_2$. Under these conditions relatively rapid water loss ensued giving very considerable wilting in from 30 to 40 hr. The drying out curves of individual leaves in a sample are shown in Fig. 6. Care was taken to keep the circulating air at bath temperature by use of large glass heating coils immersed in the bath.

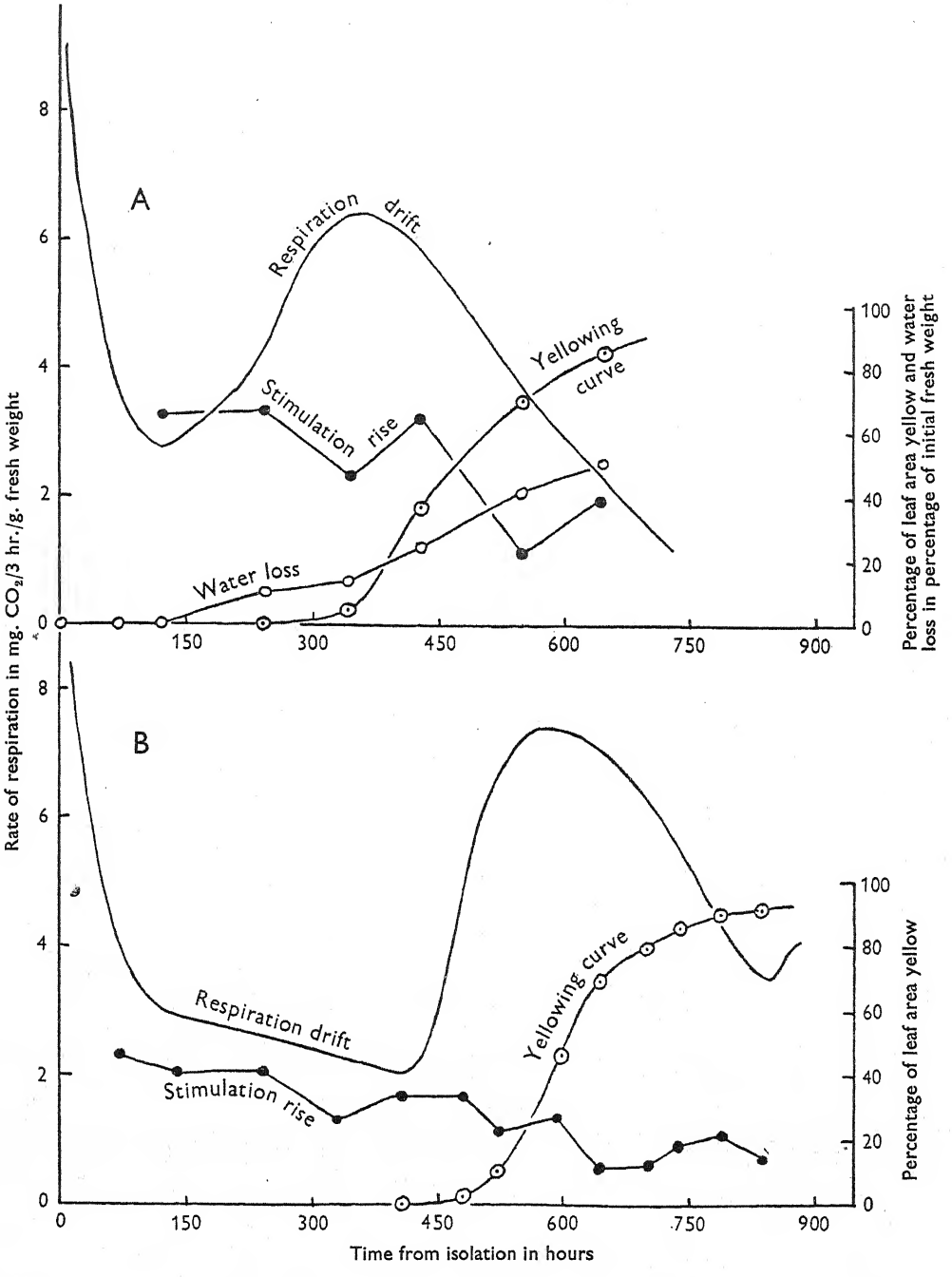

Fig. 5. Graphs showing the effects of wilting (curves *A*) on the respiration, rate of senescence and stimulation effects of starved leaves of cherry laurel. Curves for an unwilted sample are shown at *B*.

The experimental procedure consisted of following the normal starvation respiration drift of two identical leaf samples. When the protoplasmic phase of the drift was reached, circulation of dry air was started over the experimental sample and continued for about 40 hr. After this time the leaves had lost about 30% of their original weight. Circulation was then stopped and both experimental and control samples of leaves were stimulated by hand soon after. The subsequent effects on the respiration were carefully followed.

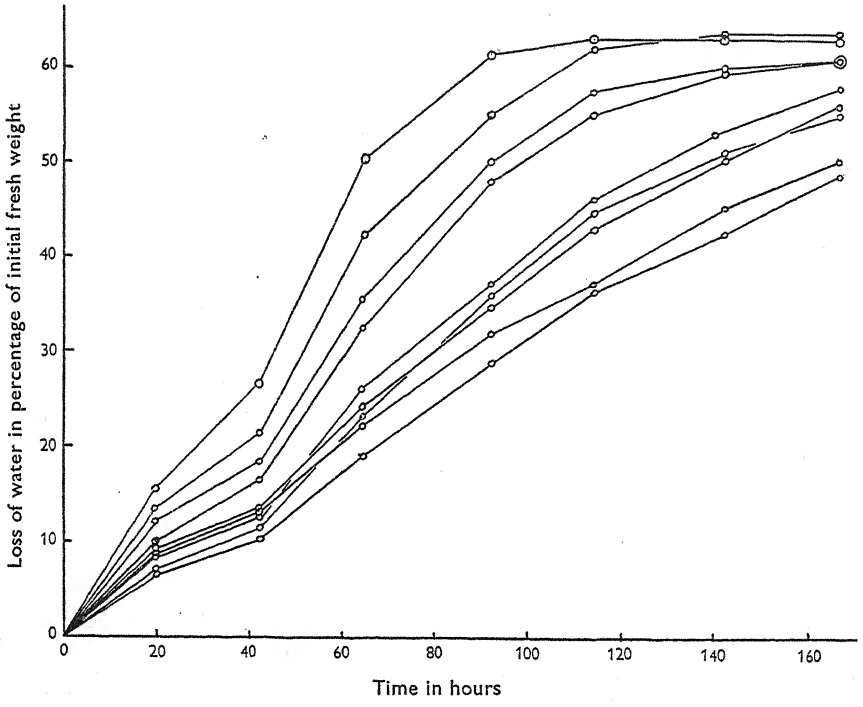

Fig. 6. Drying out curves of individuals from a sample of detached cherry laurel leaves in the dark and in a current of $CaCl_2$-dry air of 30 l./hr.

Results of a typical experiment are shown in Fig. 7. Rapid loss of water from the leaves resulted in a general raising of the drift level of respiration above normal at the same temperature, and stimulation of the leaves under these conditions resulted in a correspondingly large effect. The larger effect in this wilted sample may be due merely to the higher level of the unstimulated respiration rate. This is borne out by the fact that the ratio of the stimulation rise (height of peak of effect above the unstimulated level) to the unstimulated respiration rate is roughly the same in both cases. These results and the results of two exactly similar experiments are shown in Table 1.

Table 1

Water loss in percentage of initial fresh weight	Stimulation rise / Unstimulated rate	
	Wilted	Control
31·2	1·66	1·54
43·0	0·60	0·65
28·3	1·02	1·32

These results suggest that the reaction of the wilted leaf to mechanical stimulation is identical with that of the normal leaf, the rather larger effects obtained in the wilted samples being due entirely to a general effect on the basic level of respiration in the leaf. The different behaviour of the turgid and wilted tubers shown by Barker is most probably due to differences in the intensity of stimulus received by them, i.e. the degree of cell deformation. In the foliage leaf however the cell is presumably sufficiently deformable when in the turgid condition to give the maximum response, and variations in cell turgidity have no obvious effect on this response.

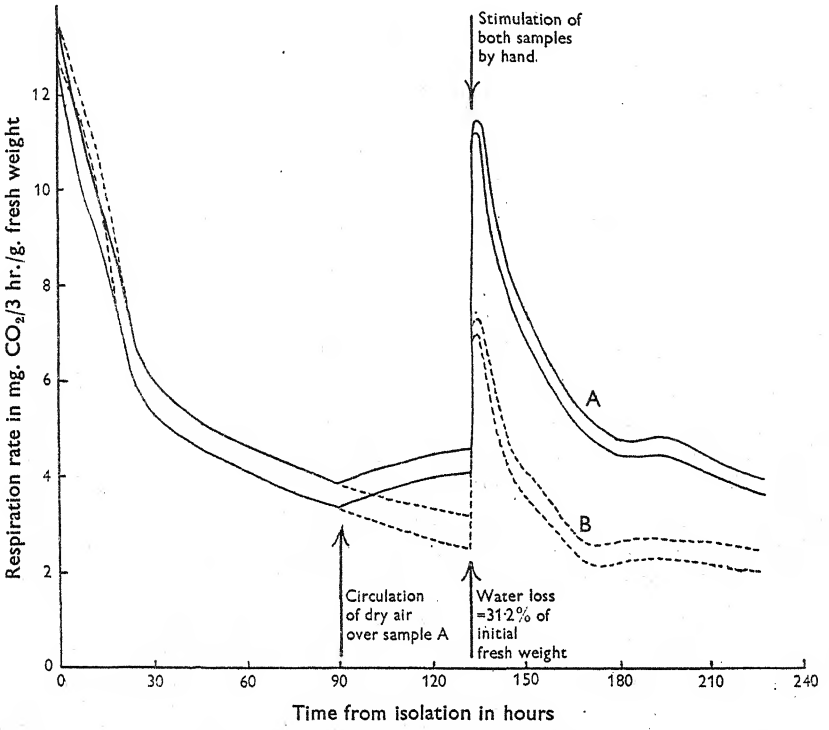

Fig. 7. Graphs showing the effects of rapid wilting on the normal respiration drift and responses to stimulation of detached leaves of cherry laurel. A, wilted sample; B, normal turgid sample.

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REVIEW

Plant Physiology. By MEIRION THOMAS. 2nd edition. $8 \times 5\frac{1}{4}$ in. Pp. xii + 596, with 61 figs. in the text. London: Churchill. 1940. Price 21*s*.

Degree students of botany in this country will be glad to know that this useful text-book has been thoroughly revised and brought up to date. First published in 1935 and reprinted with slight alterations in 1937, it was already beginning to date in certain chapters. A hundred pages have now been added to its original length and they deal faithfully with the advances of the past five years. Extensive revision is especially evident in the treatment of respiration, oxidizing systems and photosynthesis, but all sections have evidently been carefully reconsidered. Apart from tidying up the writing in certain respects, the general plan of the first edition has been adhered to, and its merits of outlook, selection and balance successfully preserved. Principles are still preferred to factual details, but the extra pages have allowed a rather fuller treatment in some places.

An unfortunate error has crept into the account of oxidation systems on p. 377, where anaerobic dehydrases are said to be sensitive to cyanides and sulphides, with a good deal of resulting confusion. Mr Thomas has written to me that this should have read anaerobic dehydrases "act in conjunction with other oxidation systems that are sensitive to cyanides and sulphides". Reference back to pp. 43 et seq. will also make clear the author's intention.

A new section has been written dealing with the substances consumed in respiration. The objection to the use of the definite article for *the* respiratory substrate would not appear to depend on lack of knowledge, as the author suggests, but on definition. The major substances consumed may now be named with some confidence, and it is a purely arbitrary matter at what point among their transformations respiration is said to begin. Some confusion is also caused in this section by a loose use of the word "oxidation". It is pointed out that fructose is more readily oxidized in vitro than glucose and this is compared with its apparently readier "oxidation" in respiration. Such a comparison is, however, very misleading, and indeed the author himself points out a few lines further on that the sugars are only the source of oxidizable substrate, their own capacity for direct oxidation being therefore irrelevant.

These small matters are not pointed out with a view to disparagement, but only in the hope of saving a few headaches among the very numerous students who in the next few years will in all probability be studying this book.

W. O. JAMES

MAY, 1941 VOL. 40, NO. 2

THE WATERMARK DISEASE OF WILLOWS

II. PATHOLOGICAL CHANGES IN THE WOOD

BY GEORGE METCALFE
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(With Plate 1 and 4 figures in the text)

INTRODUCTION

THE presence of the bacterial flora associated with watermark disease (Metcalfe, 1940) leads to two important histological changes in the wood. First, there is an early and abnormal degeneration of the protoplasm of certain ray cells, resulting in the death of the cells: secondly, tyloses appear in the vessels. An attempt to ascertain the cause of these changes is described in this paper.

As a result of the abnormal protoplasmic degeneration (henceforth referred to as "oily degeneration") the affected cells come to contain abundant oil, both in close association with the cytoplasm and as separate globules within the vacuoles. The contents of the degenerate cells are coloured brown. The colour is light at first but later becomes darker; in this condition the cells cannot be plasmolysed and are probably dead.

DISTRIBUTION OF THE DEGENERATE CELLS

General distribution. In studies of several bacterial diseases of plants, it has been observed that parenchymatous cells in the infected regions undergo oily degeneration in the same way as ray cells in watermarked willow wood. In addition, my colleagues have shown me such cells associated with many fungal diseases. In the willow such cells are associated with any local infection, such as may occur near a wound, particularly in roots. Many cells in leaves undergo this type of degeneration just before leaf-fall. It thus seems that this type of degeneration may be a general phenomenon representing one way in which living cells react to a greatly disturbed physiological environment.

In watermarked wood. When a vessel becomes occluded by bacteria all the ray cells in the neighbourhood undergo oily degeneration. In spring, in an infected branch, the bacteria establish themselves in the vessels in the outer part of the previous year's annual ring (cf. Part I) and all the ray cells in this region are affected. The ray cells in the inner part of the annual ring are not affected and degenerate normally. In the intermediate region pathological cells and healthy cells are intermixed without a definite arrangement pattern. Later in the summer the initial parenchyma and the innermost ray cells of the current year's annual ring may be affected.

During the winter, unaffected ray cells in the diseased annual ring may store

starch, but in the following spring the annual ring is invaded by secondary organisms and the unaffected ray cells become brown coloured and can no longer be plasmolysed.

NORMAL LIFE CYCLE OF A RAY CELL

In order to determine whether the appearance of oil in any particular ray cell is due to pathological causes it is necessary to know the changes in quantities and kinds of reserve substances in a similar healthy cell throughout the year. In general, throughout the autumn and winter, the ray cells in the outermost annual ring contain enormous numbers of small starch grains. In each ray the outermost cells may contain large numbers of small oil globules in addition to the starch; the inner cells may also contain oil globules but in smaller numbers. In the next oldest annual ring most, but not all, of the ray cells contain abundant starch grains. In older annual rings the number of starch-containing cells decreases rapidly and progressively with the age of the annual ring. In cells in these rings a few large oil globules may be found.

During periods of extreme cold weather the amount of oil increases, particularly in the outermost ray cells. Early in the spring the starch and oil reserves in the rays are greatly reduced, and until the autumn the ray cells are free from reserve substances in any quantity.

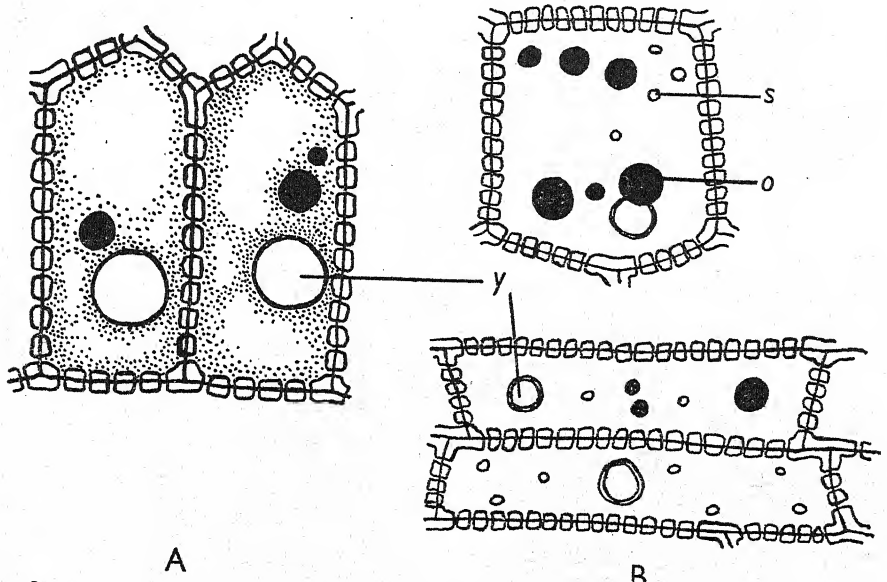
Following the hydrolysis of starch in spring, the outer ray cells are still "immature", containing large amounts of cytoplasm with two or three small vacuoles. The changes which then occur follow the usual course. Stained with iron haemotoxylin, the nucleus at first appears to have a granular structure, but it rapidly loses its ability to be stained and finally cannot be distinguished with any nuclear stain. The vacuoles fuse to give a large central vacuole and the cytoplasm decreases in amount; while this is happening a few oil globules may form in the cytoplasm and pass into the vacuole.

In the "mature" condition the cell (Text-fig. 1) has a thin layer of cytoplasm around the walls, embedded in which is a large clear globule, which will be called the *Y*-globule and which is described below. Such cells may remain alive for 5 or 6 years (as shown by plasmolysis experiments) and starch appears in them during each successive winter, but there is no further change in the organization of the cells. After about 3 years, however, a large proportion of the ray cells cannot be plasmolysed and cease to store starch; the cytoplasm disappears but the *Y*-globule usually remains in the cell (Text-fig. 1).

The Y*-globule.* The *Y*-globule appears with constancy in all fully mature healthy cells and in all pathological cells (Text-fig. 3). It appears to be bounded by a distinct membrane and is at first associated with the cytoplasm, but when this finally disappears it remains in the cell. It does not give the staining reactions of oil, but after immersion in chloroform for 12 hours it can be stained with osmic acid. In ether it bursts, leaving a shrivelled membrane, the contents being ether soluble. In Eau de Javelle the membrane is ruptured and the contents escape as small droplets which give the staining reactions of oil and are soluble in the fat solvents. The *Y*-globule thus appears to be an integumented oil globule. McLuckie & Burges

(1932) record that, in the cortical cells of *Eriostemon Crowei* F.v.M. showing mycorrhizal infection, oil globules occur which, at a late stage in their history, appear to have a membrane. This membrane stained with osmic acid, but the membrane of the *Y*-globule does not stain with osmic acid.

As the *Y*-globule does not appear until after the "disappearance" of the nucleus, its constant presence in all old cells suggests that it may be directly formed from the nucleus. In general, nothing is known about the fate of the nucleus when the chromatin can no longer be stained.



Text-fig. 1. (A) Mature ray cells, showing the *Y*-globule. (B) Old ray cells, with no remaining cytoplasm. *y*, the *Y*-globule; *s*, residual starch grains; *o*, oil globules.

Oily degeneration of ray cells

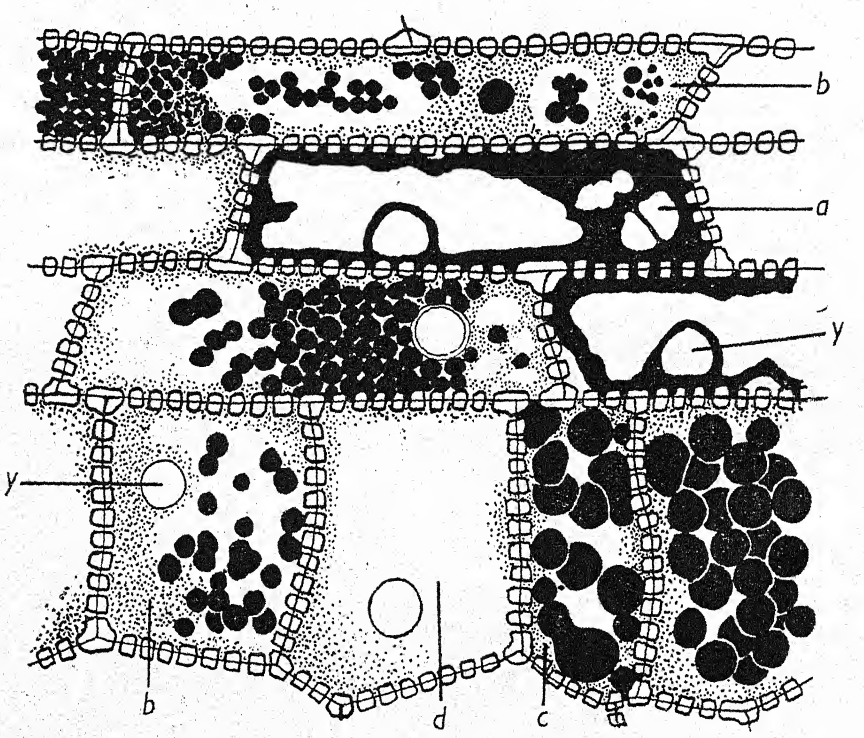
In diseased wood the ray cells in the inner part of the diseased annual ring may be sufficiently removed from bacterial influence to permit the normal degenerative processes to take place, but in the outer part of the ring the changes subsequent to starch hydrolysis may roughly be classed as following one of three courses.

Generally no further disappearance of cytoplasm occurs but changes occur causing it to mass together around the sharply-defined vacuoles and to assume a fairly homogeneous texture (Pl. 1, fig. 4). At this stage the cytoplasm often stains deeply with osmic acid. Countless small globules of oil may appear in the vacuoles. Usually the globules do not coalesce, but if they do the cell contents appear almost homogeneous. This type of pathological degeneration is very common in both types of ray cell.

In other cells large oil globules are formed in the cytoplasm (Pl. 1, fig. 1) and these pass into and accumulate in the vacuoles. The cytoplasm disappears except for a thin layer around the wall, and when this has happened the cell is very nearly

filled with oil globules which are often quite large (Pl. 1, figs. 2, 3; in Text-fig. 2*c*). The oil globules may become coalescent. This type of degeneration is frequently found in upright ray cells.

Other cells lose the greater part of their protoplasmic contents, and such cytoplasm as remains forms sharply-defined masses, often in association with the *Y*-globule, as shown in Text-fig. 3. The cytoplasm may be finely granular or homogeneous, and may or may not stain with osmic acid. Apart from the *Y*-globule, oil globules are usually few or absent. This type of degeneration is very common in the



Text-fig. 2. Typical group of degenerate ray cells as seen when stained with osmic acid. *a*, vacuoles sharply defined, cytoplasm gives the staining reaction of oil; *b*, cytoplasm disappearing, oil globules accumulating in the vacuoles; *c*, cells nearly filled with large oil globules; *d*, cell containing only the *Y*-globule and traces of cytoplasm. *y*, the *Y*-globule.

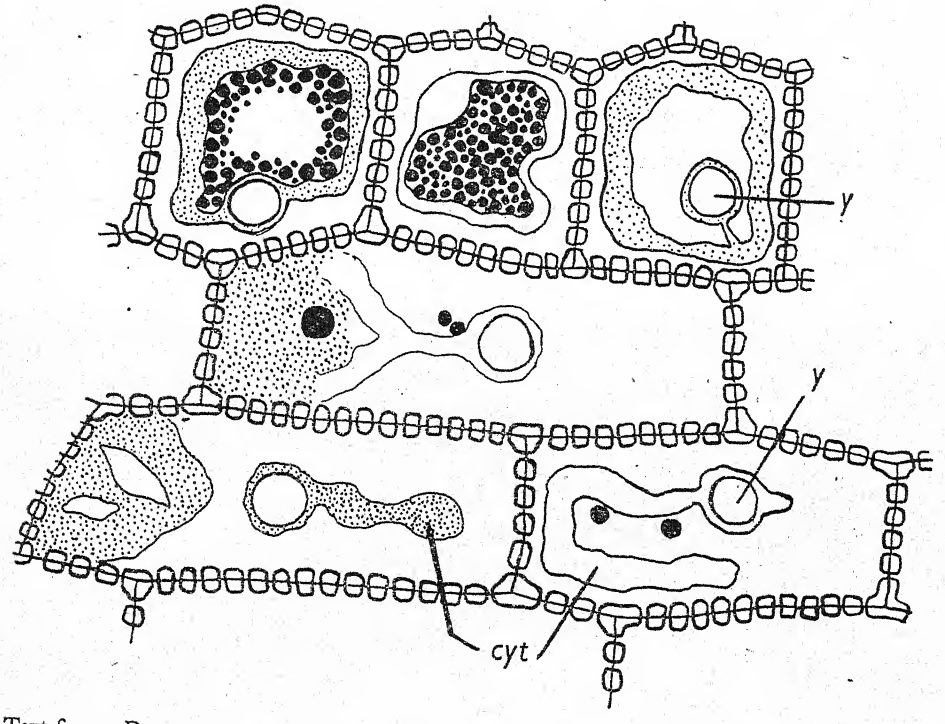
procumbent ray cells. In a very long procumbent cell, one end of the cell may degenerate in one way and the other end in a different way.

Other cells lose the greater part of their protoplasmic contents, but the degeneration processes scarcely differ from those in the "normal" degeneration of cells in healthy wood. None of the contents of these cells ever give the osmic acid stain.

In each case the contents of the cell inevitably become coloured with a brown staining-substance; this discoloration may occur at any stage of degeneration. The coloured cells do not store starch, cannot be plasmolysed, and no further change seems to occur in their contents; they are probably dead. The brown compound

seems to exert a protective influence on the cell contents by rendering them insoluble in ether and chloroform, and it often interferes with the osmic acid stain.

There is no definite arrangement pattern amongst the various types of degenerate cell, and neighbouring cells may degenerate in the same way or in different ways. The upright cells, particularly those in contact with vessels, are usually the first cells to degenerate (in the middle wood of the annual ring the upright cells bordering upon vessels are usually the only cells which degenerate).



Text-fig. 3. Degenerate ray cells, containing irregular masses of cytoplasm which may be granular or clear. The *Y*-globule is usually associated with cytoplasm. *cyt*, cytoplasm; *y*, the *Y*-globule.

Possible causes of oily degeneration

Two of the three types of abnormal degeneration described above are characterized by the mobilization of the cytoplasm of the affected cells. This proceeds beyond the point at which the normal protoplasmic organization of the cell is lost and thereafter suggests autolysis. The mobilization process is accompanied by an increase in the oxidative activity of the cell (see below) and the appearance of the brown staining substance. The latter event is accompanied by the death of the cell. There are thus two important processes going on in the cell—one of mobilization and one of immobilization—and in each case the final form of the cell contents must depend upon how far the first process has gone before the second process becomes dominant. The second process invariably accompanies the first and arises indirectly out of it,

one of the products of protoplasmic mobilization being the oxidation base, the presence of which enables the second process to take place.

The distribution of pathological cells in the rays suggests that possibly degeneration is initiated by substances which diffuse from the vessels containing bacteria, but the mobilization of the contents of the ray cells may possibly be initiated by the great demands which the bacteria make upon their physiological environment.

None of the bacteria produces an endotoxin in the specialized bacteriological sense, but it is possible that the products of bacterial metabolism are the diffusible substances which affect the ray cells. From a consideration of the substances present in the vessels, such metabolic by-products might be nitrites, ammonia, and the acids produced by the fermentation of glucose, together with other substances less easy to determine. In the fermentation of glucose by *Bacterium coli*, Harden (1901) found that the principal products were lactic, acetic and succinic acids and alcohol. These substances were recognized by analysis amongst the products in the fermentation of glucose by the four willow bacteria. It is thus extremely probable that these substances are found in the vessels in spring.

Newly-cut blocks of living willow wood were allowed to stand for a week in solutions of ammonium lactate, succinic and acetic acids, ammonia, and potassium nitrite. Other blocks were allowed to stand in filtered culture solutions (of very varied chemical composition) in which the bacteria had been grown for varying times. In the solutions of chemicals, a brown stain appeared in the ray cells which were exposed to air on the surface of the wood. With succinic acid the brown colour was also present in the ray cells within the block, and when cut transversely this block showed a discoloration closely resembling a watermark. Further, the contents of the ray cells had undergone oily degeneration and large quantities of oil had accumulated in many of them. In the filtered culture solutions the blocks appeared to be unaffected.

It is thus possible experimentally to induce oily degeneration, with the accompanying brown colour, using a product of bacterial metabolism. It must be pointed out, however, that the solutions used were relatively concentrated (1 % by weight) and that it is improbable that such concentrations are reached in the vessels. When the experiment was repeated, using diluted solutions, very inconclusive results were obtained.

If the oily degeneration is caused by bacterial products it is difficult to understand why some cells are affected and some cells unaffected. Moreover, as has been stated above, certain cells undergo oily degeneration in leaves on healthy trees, particularly prior to leaf-fall.

It is difficult to decide whether the presence of the bacteria causes mobilization of the reserves of the ray cells. Guilleman & Larson (1922) have shown by analysis that in *B. coli* (which is related to *B. salicis* and *B. aerogenes*) the total "ash" in the cells is 1·45 % of the fresh weight; of this total ash, 20·7 % is phosphorus (estimated as element). Bacteria with a phosphorus content of this order must, during active growth, take a considerable toll of the phosphorus salts in the vessels, and it is possible that the high demand leads to the rapid mobilization of the phosphorus

component of the lecithins of the ray cells, resulting in an accumulation of oil. Often, however, the amount of oil formed in a cell is too great to have been derived solely from the protoplasm of that cell, and in these instances it must be assumed that transportation of substances into the cell occurs; probably these substances come from neighbouring cells.

The brown compound

Day (1924) concluded that the brown stain is formed as a result of an oxidation process. If a recently diseased branch is cut in summer the waterlogged region turns scarlet red in colour, turning to brown and finally to black. This sequence suggests the formation of a melanin. In an intact branch the colour change is more gradual, resulting in a dark brown colour in the ray cells.

The oxidation substrate. It was found that if stained wood was boiled with water for half an hour the brown compound was completely extracted from the wood, leaving it white. A red liquid was obtained which gave the general biochemical reactions of catechol tannins. A control experiment showed that catechol tannins were present in healthy wood but in much lower concentrations.

The abnormal distribution of tannins in diseased wood was studied by treating freshly cut sections of diseased and healthy wood with reagents. It was found that in diseased wood there was a considerable concentration of tannin compounds in the ray cells in the outer part of the waterlogged region (where the stain in the ray cells was still very light brown). The cells in the rest of the stained region contained tannin compounds but not in the same quantities. When similar sections were treated with concentrated hydrochloric acid a deep red colour developed rapidly in the ray cells in the outermost waterlogged region, but more slowly in the rest of the wood. The red colour was due to the formation of phlobaphenes, which are formed by the oxidation of the phloroglucinol compounds produced by the hydrolysis of catechol tannins.

It thus seems probable that the oxidation substrate involved in the formation of the brown compound is a catechol tannin or, more probably, a polyphenolic breakdown product. If blocks of healthy wood are allowed to stand for a few days in a very dilute solution of catechol the blocks turn black on the exposed surfaces; the centre of the block does not stain until the block is split open and exposed to the air, when it stains very rapidly. The black colour is due solely to the appearance of a brown stain in the ray cells. Thus an increase in the concentration of catechol in the cells of healthy wood leads (with a sufficient oxygen supply) to the appearance of the brown colour.

Enzymes. The abnormal distribution of oxidative enzymes in diseased wood was studied by immersing sections of healthy and diseased wood in guaiacum solution. Peroxidases are particularly abundant in the stained and waterlogged parts of diseased wood. Oxidases are extremely abundant or extremely active in the waterlogged area immediately outside the ring of diseased vessels but are absent or inactive in the inner stained regions. The oxidase system required for the formation of melanin is present in the outermost waterlogged region; in sections which

had been standing in tyrosine solution for several hours a black colour appeared in the ray cells in this region, and with *p*-cresol cells in this region gave an intense orange colour. Under natural conditions melanin is not formed in diseased wood, and tests failed to show the presence of tyrosine in this region, but, as stated above, when a cut branch is exposed to the air a series of colour changes result typical of the melanin formation from tyrosine.

The oxidase system of each of the four bacteria was investigated. All the bacteria produce an extracellular peroxidase, but apparently all lack the indophenol oxidase. The increased oxidase activity of the outer diseased wood must therefore be caused by the ray cell enzymes and not by the bacterial enzymes.

Diseased wood was characterized by a marked dehydrogenase activity. When the bacteria were tested separately it was found that glucose and succinic dehydrogenases were present in all four species of bacteria, and had been excreted into the culture fluids in which the bacteria had been grown. *B. salicis* did not possess lactic dehydrogenase; this was present in the other three bacteria but not in their culture fluids.

Thus in the outermost waterlogged region, which is only lightly stained, there is present not only a substrate which can be oxidized but the enzymes necessary for the oxidation, and it would seem that shortage of oxygen is the factor which prevents rapid oxidation taking place. In the inner wood, where the colour is darker and oxidation presumably more complete, part of the oxidative system is inactive.

DISCUSSION

It is now widely believed that the presence of polyphenolic substances in the tissues of a plant may confer a considerable degree of disease resistance upon those tissues, and it has become common to regard the appearance of such compounds in the cells of a diseased plant as a type of defensive response of the plant to invasion.

Dufrénoy (1936), from a careful study of a large number of plant diseases, has given a generalized description of the reactions of a parenchymatous cell to the presence of a parasite. Such a cell may react in one of two ways: (*a*) the cell reserves are mobilized, starch and complex proteins being changed into simpler compounds; the plastids revert to the mitachondrial condition and the large vacuoles become partitioned off by a network of cytoplasmic strands into smaller vacuoles; the cell thus returns to the embryonic condition and may resume division; (*b*) the presence of the parasite, or the death of neighbouring cells, alters the metabolism of cells so that phenolic substances (mostly tannins of the gallic group) accumulate in the vacuoles making these cells uncongenial for the pathogen. These changes thus represent a defensive mechanism against further attack by the parasite.

Dufrénoy has worked chiefly with intracellular parasites (fungi and viruses), and it is reasonable to expect that the cellular changes that such organisms initiate may differ from the changes initiated by intercellular pathogens (e.g. most phytopathogenic bacteria). With an extracellular pathogen it is difficult to regard changes, such as those described by Dufrénoy, in the organization of the cell as a defence mechanism; certainly the accumulation of polyphenolic compounds in the ray cells of

diseased willow cannot be interpreted in this way, partly because such changes can occur in cells far removed from the parasites, partly because similar changes can occur (under unfavourable physiological conditions) in cells in tissues free from disease. It is probable that the accumulation of oil and polyphenolic compounds, and the increased oxidative capacity of the tissues (which results in the oxidation of the polyphenolic compounds to brown substances) represent a normal and fundamental reaction of cells to a greatly disturbed physiology; any properties of disease resistance which may be conferred by these changes are merely a fortunate accident.

Tylosis formation

In a diseased annual ring, tylosis formation takes place locally in the region where the vessels are infected; in tissues free from infection tyloses are seldom observed. In the spring, during the first invasion of the wood by bacteria, tyloses appear sporadically but many more appear during the winter. Very few appear during the invasion of the wood by secondary organisms, possibly because most of the ray cells in contact with vessels have, by that time, undergone oily degeneration and are dead.

Tyloses form into vessels whether the vessels contain bacteria or not. The bacteria are compressed into compact masses between the tyloses; later in the season the bacterial masses become brown stained and in this condition they were found to be non-viable.

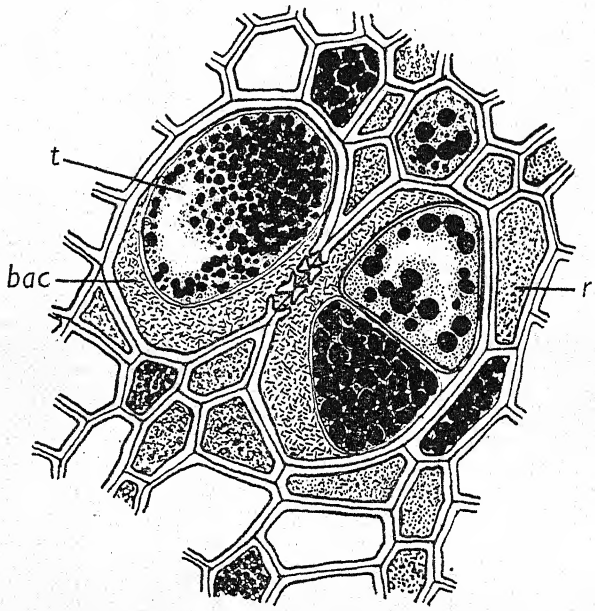
The protoplasmic contents of the tyloses undergo the same type of oily degeneration as do the contents of any ray cell in an infected region, and in a similar manner become brown-stained. As the thin tylosis wall is often closely adpressed to the vessel wall, vessels containing such tyloses appear to have granular or gum-like brown contents when examined in transverse sections. Degeneration of the contents may occur before the tylosis is large enough to occlude the vessel; such tyloses do not increase further in size (Text-fig. 4).

The cause of tylosis formation. The presence of tyloses in diseased plants has led many investigators to the conclusion that they are formed as a result of the stimulation of the ray cells by the metabolic products of the parasites. Such a view was expressed by Smith (1920, p. 477). It is established, however, that tyloses can form in sterile wood, particularly after wounding.

Experiments were conducted to ascertain whether bacterial products can cause tylosis formation in willow wood. Lengths of branch were cut under water to prevent the entry of air into the vessels, and were allowed to stand in solutions of succinic acid, acetic acid, alcohol, formate, potassium nitrite, bicarbonate and ammonia of various strengths, and also in filtered bacterial culture fluids. After 14 and 21 days the wood was examined for tyloses but none were found. Thus the products of bacterial metabolism in artificial media play no direct part in tylosis formation.

Klein (1923) concluded that the factor responsible for the initiation of tylosis formation is the entry of air into the vessels; so long as vessels contained water only, no tyloses formed. Ebes (1938) made a thorough study of tylosis formation in the

red beech. The water content of the wood was found to be the most important controlling factor. Blocks of wood were slowly dried until they had lost a certain proportion of their water content; the wood was then kept in a constant humidity chamber, some in contact with water, some not. Wood which had lost 25 % of its water content formed tyloses only if not in contact with water; with 50 % water loss tyloses formed only if the wood was in contact with water. With 60 % water loss or more, no tyloses formed. Ebes considers that these results support Klein's opinion of the cause of tylosis formation.



Text-fig. 4. Vessels containing bacteria and tyloses. The protoplasm of the tyloses has undergone oily degeneration. *bac*, the bacteria; *t*, a tylosis; *r*, ray cell.

Experiments, using Ebes's technique, showed that tyloses formed in willow wood which had been slowly dried to about 30 % water loss and then kept for a week in a damp chamber. Examination of the wood showed that many vessels, and in particular those which contained tyloses, were full of air bubbles. In wood not subjected to such previous drying no tyloses formed.

In watermarked wood, tyloses are found in the vessels immediately the wood is cut. In wood with tyloses many vessels are filled with gas; this gas is probably not air but the mixture of gases produced by the fermentation of dextrose by the secondary bacteria. It may be that the presence of such a gas mixture in the vessels has the same effect on the ray cells as the presence of air; if this is so it may explain why tyloses are frequently found in unwounded diseased plants whose vessels can never have contained air.

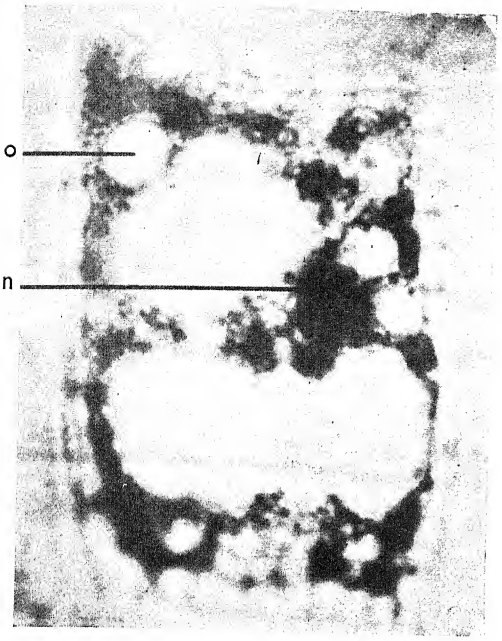


Fig. 1

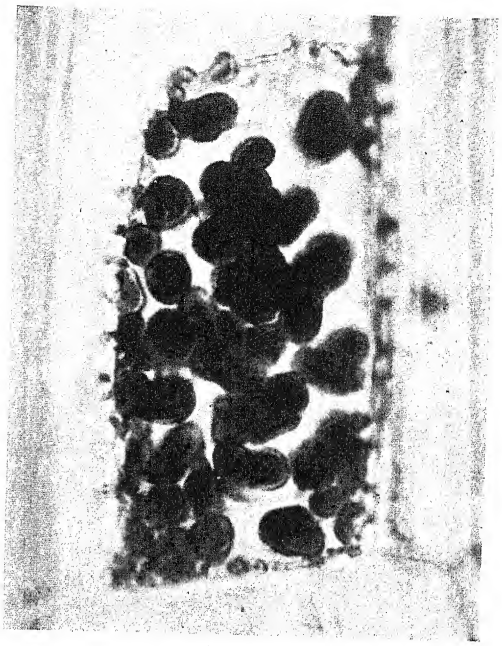


Fig. 2

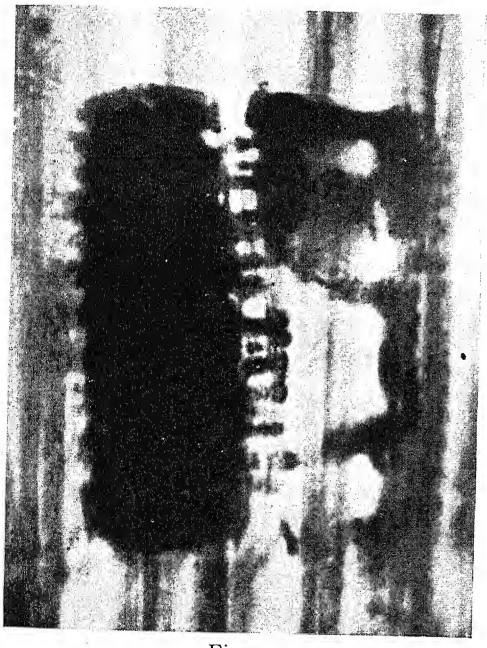


Fig. 3

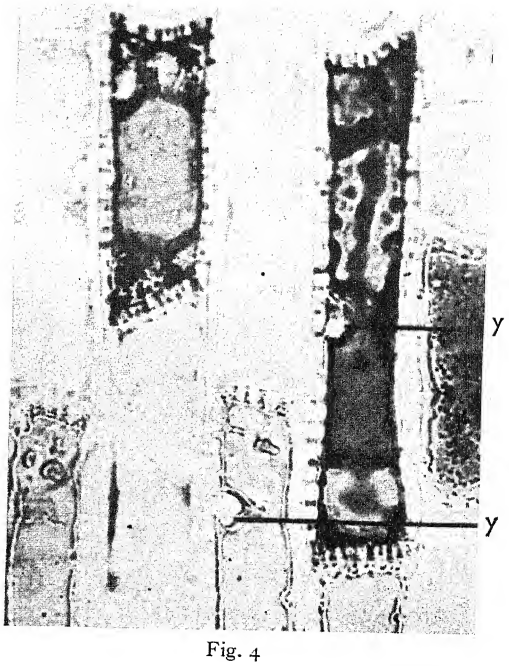


Fig. 4

SUMMARY

1. An abnormal type of protoplasmic degeneration is described; this abnormal degeneration occurs in the ray cells of watermarked willow wood and in many other tissues, diseased and healthy. It is characterized by the appearance of large amounts of oil in the cell and also of a brown coloration.

2. The degeneration is probably a usual result of a greatly disturbed physiology which in this case is caused by the presence of the bacteria. The brown stain is an oxidation product of catechol tannins or their breakdown products.

3. The initiation of tylosis formation is connected with the presence of gas in the vessels.

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EXPLANATION OF PLATE 1

Fig. 1. Ray cell stained in iron haemotoxylin, showing the formation of oil globules in the cytoplasm—an early stage in oily degeneration. The vacuoles are sharply defined. *o*, oil globules; *n*, the nucleus.
Fig. 2. Degenerate ray cell, stained in osmic acid, containing numerous oil globules but no cytoplasm.
Fig. 3. Ray cells stained in osmic acid. One cell is nearly filled with oil globules, in the other the cytoplasm is disappearing.
Fig. 4. Group of ray cells, stained in osmic acid. Some cells are almost devoid of contents, in others the cytoplasm gives the staining reactions of oil and the vacuoles are sharply defined. *y*, the *Y*-globule.

STUDIES OF THE POST-GLACIAL HISTORY OF BRITISH VEGETATION

VI. CORRELATIONS IN THE SOMERSET LEVELS

By H. GODWIN
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(With 8 figures in the text)

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Introduction

In 1936 and 1937 the opportunity presented itself through the kindness of Mr St George Gray and Dr A. Bulleid of obtaining pollen-analysis sequences in relation to a number of archaeological horizons in the neighbourhood of the village of Meare, Somerset, and later Mr H. S. L. Dewar assisted a similar correlation near the Somerset coast at Combwich. The results of these investigations do not do more than indicate an opening into a very large field of enquiry, but they will be seen to have a certain consistency in themselves, and to provide some conclusions of general importance to knowledge of the post-glacial history of this country.

The Somerset Levels is an extensive lowland area bordering the southern side of the Severn estuary (Fig. 1). In the course of the great post-glacial marine transgression an irregular depression between the Cotteswolds, Mendips, Poldens, and the Quantock Hills became filled with alluvial deposits of clay, peat and silt. Over the flat surface of the plain thus made the rivers Axe, Brue, and Parrett with their tributaries meandered to the sea, and the plain ended seawards in a low coastline marked merely by lines of dunes and the river estuaries. In origin, therefore, we may regard the Somerset Levels as the western homologue of the East Anglian Fenland, and may reasonably expect not only that the type of investigation fruitful in the Fenland will be profitable here also, but that comparison of the two regions may have its own intrinsic value.

As in the Fenland and the coastal region of north-western Germany already compared with the Fens, a primary division of the region is immediately evident into the coastal clay or silt region, and the hinterland where the alluvial deposits are

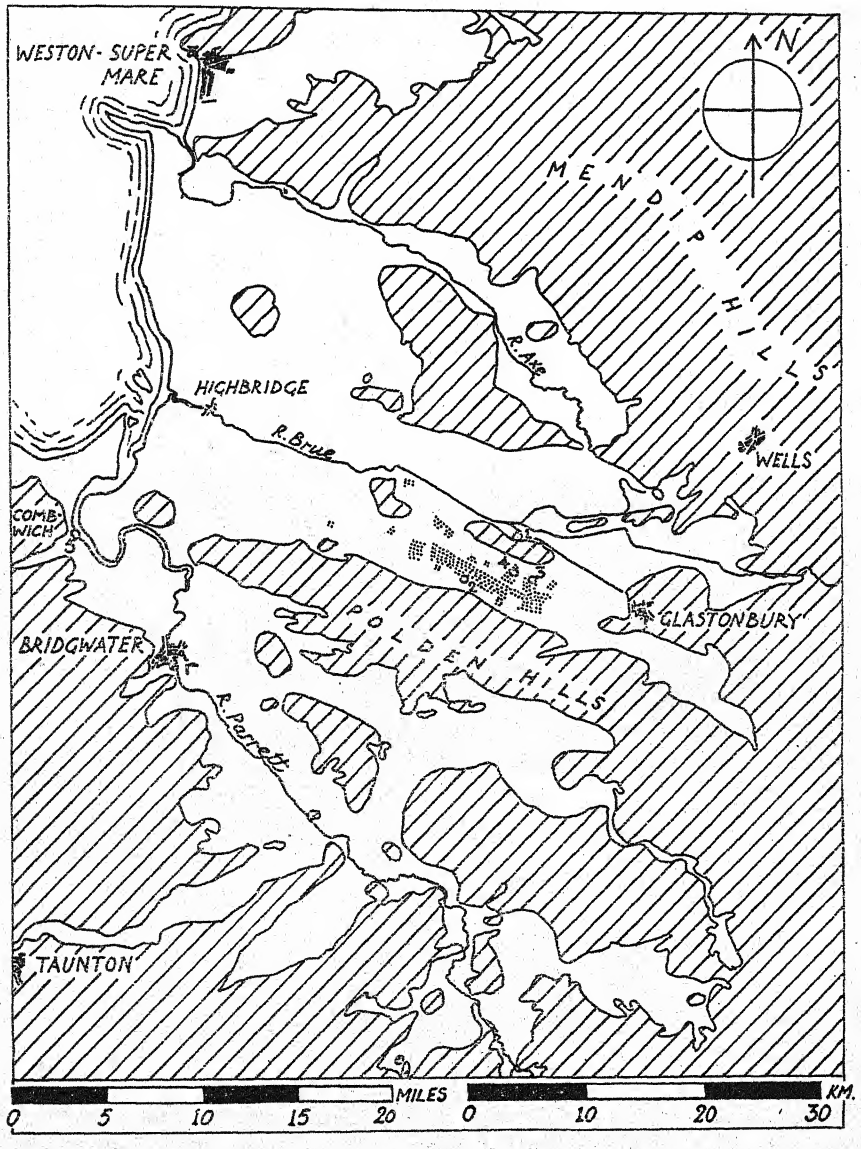

Fig. 1. Map showing the greater part of the Somerset Levels. The dotted areas are relict raised-bogs, and the numbered circles are sites mentioned in the paper: (1) Meare Lake Village, (2) Shapwick Heath Roman hoards, (3) Meare Heath I, (4) Meare Heath II, (5) Combwich.

almost entirely peat. The coastal belt of clay has a general surface level about equal to that of present mean spring tide high-water (about 18–20 ft. O.D.), it is several miles wide, supports an intensive agriculture, and is dotted with brick-pits. The town

of Bridgwater which stands in this clay land astride the River Parrett recalls at once in all its major characteristics the town of Wisbech on the natural estuary of the Great Ouse in East Anglia. Behind the clay land is a great extent of peat country now given over to pasture, osier growing, etc. It is intersected by innumerable drains (locally called rhynes) and the natural river channels, lost as in the Fens, have not yet been investigated or indeed even recognized. At the same time descriptions by Dr Bulleid (1933) of meandering clayey ridges in the peat areas leave little doubt that 'roddons' are present here as in the Fenland, and recording their disposition, structure and age would be a physiographic study of great interest. The river systems drain much highly calcareous country, and the rhynes are full of the typical vegetation of fens, but whereas in the East Anglian Fenland true raised-bogs have developed above the fen peat in one region only (Woodwalton, Holme and Yaxley), in the Somerset Levels very extensive raised-bogs formerly existed. None now remains in an active growing state, but the map given by Moss in his admirable survey of the vegetation of Somerset (Moss, 1907) shows a number of uncultivated 'heaths' which represent the dried surfaces of such bogs (see Fig. 1). Until recently a few characteristic species lingered alive upon them, but in any event the peat and bog stratigraphy allow no doubt that these mires were originally convex ombrogenous[1] bogs raised by the accumulation of *Sphagna*, *Calluna*, *Scirpus*, *Eriophorum*, *Molinia* and associated species. The last persisting group of these raised-bogs includes the areas known as Shapwick Heath, Meare Heath, and Sharpham Heath. Hereabouts peat cutting is going on in an extremely extensive and intensive manner, so that before long all traces of these bogs will have vanished, and with them valuable keys to our country's post-glacial evolution. It is extremely likely that similar raised-bogs were formerly very abundant in the peat areas of the levels: in the big area north of the 'island' of Meare, the cultivated ground is clearly derived from *Sphagnum* peat, the east lake village at Meare is underlaid by such peat, and traces of it could probably be found in many other sections of the levels. When the peat cuttings are deep they are invaded by the calcareous drainage water of the present river system, so that the cuttings are full of typical eutrophic[1] species (*Typha*, *Phragmites*, *Alisma*, etc.), although the peat banks of the cutting bespeak an origin from severely oligotrophic[1] communities.

STRATIGRAPHY OF RAISED-BOGS

(*a*) *Shapwick Heath*

During the peat cutting several interesting archaeological discoveries have been made, and it has proved possible in some instances to learn with fair accuracy the position of the find in relation to remaining peat deposits.

In May 1936 on Shapwick Heath a peat digger discovered a small hoard of

[1] *Ombrogenous* bogs are those which form under direct influence of local precipitation and evaporation without dependence upon or influence by drainage water from outside. They receive only small mineral supplies as dust, etc. from the air, and therefore become very poor in these substances, supporting only species of low mineral requirement, *oligotrophic* species. By contrast *eutrophic* species generally develop in fenland or similar areas when access of drainage water maintains high concentrations of mineral salts.

Roman objects at a point 166 yd. due south of Decoy Pool Drove, and 1200 yd. due east of the main Shapwick to Westhay road. The objects and circumstances of the discovery have been recorded by H. St George Gray (1936). A handled pewter cup was found wrapped in dry grass and enclosing a small earthenware beaker, which itself held 120 silver coins. These coins suggested a burial date of about A.D. 410. The depth of the hole containing the vessels was about 60 cm. from the present bog surface.

Under almost exactly similar conditions and only a few feet laterally from the first discovery, a second of the same type and date was made rather more than a year later (St George Gray, 1937), at a time when the author was doing field work upon the Heath. The conditions of the new find were closely noted. It has been stressed that the coins of the second hoard strongly suggest an earlier and separate interment at about A.D. 388. A third coin hoard has more recently been found close by, consisting of 1123 bronze coins buried at much the same time and depth as the last hoard, and enclosed in a damaged pewter canister (St George Gray, 1939). Even more striking discoveries followed, all very close to one another. In July 1938 a bronze bowl was found at a depth of 3 ft., and in February 1939, at a depth of 3·85 ft., a pedestalled bowl of pewter and a wooden tankard, encased in sheet bronze and having a solid bronze handle. According to St George Gray (1939) they were presumably buried, in the late Roman age, from the same surface as the coins, but to a greater depth.

In September 1936 a boring was put down about 3 ft. (1 m.) away from the site of the first Roman hoard: the stratigraphy was recorded, and pollen samples were taken. In a summarized form the stratigraphy may be represented as follows:

	cm.	
F	0– 15	Raw humus of the wood floor, tree roots, fern leaves, wood mosses, etc.
E	15–135	*Molinia-Sphagnum* peat with twigs of *Myrica* and occasional *Calluna* and *Eriophorum*.
	135–140	Fresh *Sphagnum* peat.
D	140–375	Dark brown highly humified *Calluna-Eriophorum-Sphagnum* peat.
C	375–380	Wood peat.
B	380–525	Grey-black *Phragmites* peat, with roots of *Carex*, fruits and rhizomes of *Cladium* and seeds of *Menyanthes*.
A	525–550	Soft blue clay.

Throughout the cuttings on this and neighbouring bogs a twofold division of the exposed peat may be widely seen, and is well known to the peat cutters (Fig. 2). There is an upper layer seldom more than 1 m. thick, of rather fresh peat, pale in colour, of low density and useful only for litter and similar purposes. Below this is a dark chocolate-coloured peat of high density and cheesy consistency, which is valuable as fuel, and indeed represents the highest quality of burning peat. There is little doubt that this is a very highly humified *Sphagnum-Calluna-Eriophorum* peat, although the *Sphagnum* is recognizable only microscopically. The transition between

the dark lower and pale upper peat is generally sharp and marked by a layer of very fresh pale *Sphagnum* peat, but in some places there is a transition layer of banded peat. There can be little doubt that this twofold division of the raised-bog peat corresponds with the similar division seen in peat bogs in many parts of north-western Europe, where the boundary is spoken of as the 'Grenzhorizont' and where it is generally held to correspond with the opening of the Iron Age, and to have been caused by the 'climatic deterioration' which marked the onset of the cold, wet, Sub-Atlantic climatic period after the drier Sub-Boreal. In the British Isles this 'Boundary Horizon' has not yet been very clearly correlated with any archaeological period, and it is interesting to see that it considerably precedes the fourth-century Roman surface of Shapwick Heath.

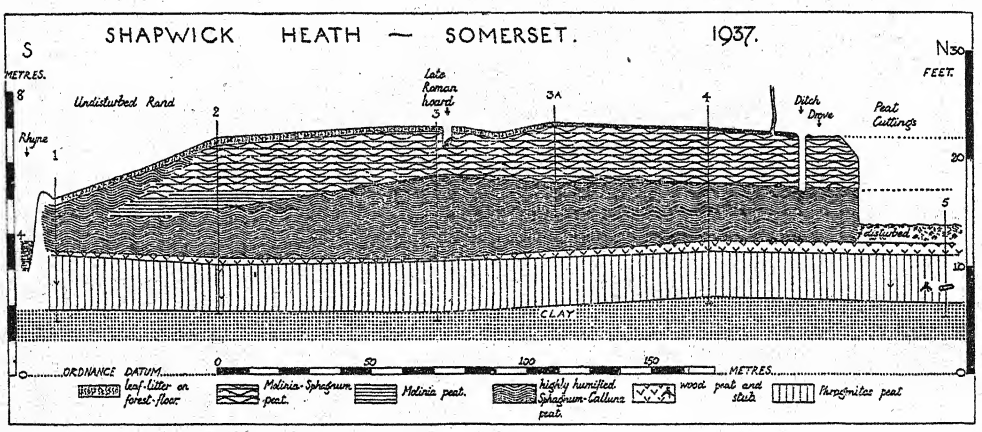

Fig. 2. Profile of raised-bog at Shapwick Heath, passing through the site of the Late Roman hoard. The vertical lines represent borings. The stratigraphy has been much simplified for the upper *Sphagnum-Molinia* peat, and also for all the region near the sloping rand. The Boundary Horizon separating the lower humified peat from the upper unhumified peat is very clear.

The Roman hoard was found within 100 yd. of the steeply sloping natural margin (the rand) of the bog, which is still uncut although densely covered with brambles (*Rubus fruticosus* agg.) or *Epilobium angustifolium*. A series of five borings in a line across the still intact part of the bog gave the data on which the profile of Fig. 2 has been constructed. These borings are too few to permit of more than a general broad statement of the bog structure, but some features emerge clearly enough. The natural curved margin indicates the original morphology of the raised-bog. The bog rests throughout on a blue clay, the upper surface of which has a fairly flat surface at about +6·0 ft. O.D. Examination of the faunal content of what appears to be the same clay, from a site 1250 yd. (1120 m.) north-westwards (see p. 129), shows the clay to have been laid down under brackish water conditions. Over the blue clay is a grey-black *Phragmites* peat, locally varying in character towards a coarse detritus peat, but always suggesting an origin close to reed-swamp vegetation. Stems and leaves of *Phragmites* are abundant in it, seeds of *Menyanthes trifoliata* are abundant, in places fine rootlets of *Carices* occur densely, and sometimes *Cladium* rhizomes can be recognized. Twigs or other wood fragments (*Alnus*

or *Salix*) occur at intervals, and in the deep cuttings at the north end of the profile there is a layer of rather abundant horizontal wood and stumps of *Betula* and *Alnus*. It is possible that traces of this layer can be found under the rest of the bog in the middle of the *Phragmites* peat. However this may be, there is an unmistakable and consistent layer of wood peat everywhere overlying the *Phragmites* peat, and clearly exposed in the deeper cuttings. It is of course the commonest type of transition at the base of a raised bog from the underlying fen peat to the oligotrophic *Sphagnum* peat, and here again the wood peat is directly succeeded by highly humified (H 8 or 9) dark brown *Sphagnum-Calluna* peat in which at intervals *Eriophorum vaginatum*, *Scirpus caespitosus*, twigs of *Calluna* or ericaceous rootlets are frequent. There is about 2 m. thickness of this decayed *Sphagnum* peat, and then a clear Boundary Horizon separates it from the upper less decayed peat. The constitution of this upper peat is extremely variable, and its structure complex, so that the few borings available give insufficient data for stratigraphic analysis. It contains throughout a great deal of *Molinia*, sometimes fresh and sometimes very mouldered, and with this often occur twigs of *Calluna* and *Myrica*, the spiky twigs of the latter species being abundant throughout the upper peat. Sometimes the *Molinia* is associated with Sphagna, and in other places the Sphagna occur with *Eriophorum vaginatum*, *Andromeda*, *Oxycoccus* and *Scirpus caespitosus*. Such *Sphagnum* peat is never so humified as that below the Boundary Horizon, and immediately above that horizon there is generally a layer 5 to 20 cm. thick of extremely fresh *Sphagnum* peat.

The two following records of the stratigraphy from positions distant from the bog margin will give some idea of the character of the upper peat:

cm.	3 A
0– 20	Litter of forest floor *Molinia* ab.
20– 30	Pure *Molinia* peat.
30– 70	Very mouldered black *Molinia* peat with twigs of *Calluna* and *Myrica*.
70– 80	Fresh unhumified *Sphagnum* peat with leaves of *Andromeda*, *Oxycoccus* and very frequent *Eriophorum vaginatum*.
80– 90	*Molinia* peat with abundant twigs and leaves of *Myrica* and some *Betula*(?). Some fresh *Sphagna* (R=4–5).
90–150	?*Molinia* peat with abundant rootlets. Twigs of *Myrica*, and occasional *Erioph. vaginatum* and *Scirpus caespitosus*.

cm.	3
0– 15	Raw humus of forest litter with living tree and fern roots, light brown.
15– 35	Light brown fresh *Molinia* (? *Sphagnum*) peat.
35– 75	Black fibrous peat with abundant *Molinia* and twigs of *Myrica*.
75– 80	*Eriophorum vaginatum* and *Oxycoccus*.
80–100	*Eriophorum vaginatum*, *Myrica* and *Calluna*.
100–130	Dark brown *Molinia* peat (H=4–5) with twigs of *Myrica*.
130–135	*Molinia* and *Sphagnum* (H=6).
135–140	Fresh *Sphagnum* peat (H=5).
140	(? Boundary Horizon).

cm.	3 A	cm.	3
150–170	Unhumified *Sphagnum* peat.	140–150	Dark chocolate brown humified *Sphagnum-Calluna-Eriophorum* peat (H = 8–9).
170	(? Boundary Horizon).		
170–270	Humified dark brown *Sphagnum-Calluna* peat.	150–375	Ditto but H = 6–8.

The bog surface is not flat, and the profile exposed in the upper peat shows large lenticular structures several metres across indicating former heterogeneity of the growing bog surface. In boring 4, *Polytrichum* and brown mosses are abundant in the upper peat, and though *Sphagnum*, *Calluna*, *Myrica*, etc., are present, there is no clear indication of *Molinia*, and at the base there are remains of *Carices* and *Equisetum*. Borings 1 and 2 reflect the special conditions of the bog margin, and in bore 1 it is not possible to trace the division between the upper and lower peat beds.

Parts of the bog surface still bore in 1936–7 an open woodland of birch, oak and pine with sallow and sweet-gale undergrowth, although this had been cleared immediately before peat cutting at the site of the profile. The litter of this forest floor covers the bog surface completely.

The bog stratigraphy is interesting in two particular features of difference from most other raised-bogs we have seen. The first is the extreme abundance of *Molinia* in the Sub-Atlantic peat, a feature so pronounced that it presupposes at least a fluctuating dominance of the growing bog surface by Molinieta. This is not a usual condition of the British bog surfaces to-day, apart from the effects of drainage and burning, but large areas of Molinieta do occur on the living surface of the great western bog at Tregaron, Cardiganshire (Godwin & Conway, 1939). The second feature concerns the depth of the late Roman hoards. These were evidently buried, although the wetness of any raised-bog will not allow us to suppose they were put deeper than 3 or 4 ft., from the surface. The bog surface of the end of the fourth century A.D., was therefore very likely near to the position of the present bog surface, and there has been no effective formation of peat above this level. The tree-grown surface shows no signs of having had peat taken from it, and the levelled bog profile does not suggest it either. It seems most probable that peat accumulation ceased during or not long after the Roman period. It is conceivable that climatic change brought this about, and it might be said, following the lead of Granlund's conclusions on the bog development of southern Sweden, that the bog reached a degree of convexity which improved drainage to the point of inhibiting further growth of the bog. A third, and perhaps more plausible hypothesis is that drainage or cutting in the Roman period may have caused drying of the bog surfaces. We cannot choose between these hypotheses without more evidence, but we shall at any rate be aware of a problem and alert to recognize any evidence which appears.

Throughout the Sub-Atlantic peat there are remarkably abundant twigs and leaves of *Myrica gale*, but once again the explanation of this is not evident.

(*b*) *Shapwick Station* (*S.S.*)

From the site of the profile above described the derelict *Sphagnum* bog extends westwards and the main Shapwick-Westhay road crosses it. A single sample boring was taken about 10 yd. (9 m.) west of the road and 210 yd. (192 m.) south of the railway station. Although dry and grass-covered the surface appeared not to have been cut for peat, and its level was determined as +16·8 ft. O.D. The boring gave the following sequence:

cm.	
0– 90	Chestnut brown, very mouldered, dry, highly humified *Sphagnum* peat with *Calluna*.
90–100	As above but paler and less decayed.
100–130	Little decayed *Sphagnum* peat with *Calluna* and *Eriophorum*.
130	Boundary Horizon.
130–340	Highly decayed *Sphagnum-Eriophorum* peat, with *Calluna*, *Scirpus caespitosus*, *Oxycoccus* rootlets and twigs of *Myrica*.
340–380	Dark brown, highly humified hypnum-moss peat.
380–500	Soft blue clay (samples taken for microfauna determinations).

The twofold division of the *Sphagnum* peat is evident again, but the blue clay surface is somewhat lower (+4·0 ft. O.D.) than in the profile borings. The absence of a phase of reed-swamp development here is striking, and suggests the growth of a *Sphagnum* mat directly over open water.

The following reports on the content of diatoms and of Foraminifera indicate the conditions under which the blue clay was probably laid down. Samples were secured at 380–400 cm. (S.S. (1)), and 440–500 cm. (S.S. (2)).

Report on diatom content, by Chr. Brockmann.

S.S. (1), *steifer blauer Klei*, wenig Diatomeen, vorwiegend *Podosira stelliger* und *Melosira westi*. Marine Ablagerung.

S.S. (2), *steifer blauer Klei*, mässiger Diatomeengehalt, ausser Marinen auch autochthone brackische Arten, Marine Ablagerung mit brackischem Einschlag. 'Die Proben sind mehr oder weniger marine Ablagerungen. Bei der Probe S.S. (2) ist ein deutlicher brackischer Einschlag festzustellen, sodass diese Ablagerung sicher als brackischmarin zu bestimmen ist. Bei den anderen Proben wurden zwar nur marine Schalen gefunden, jedoch wage ich daraus noch nicht den rein marinen Charakter dieser Ablagerungen abzuleiten. Sicher ist nur das es sich um Bildungen im Bereich der Tiden handelt.'

The list of diatoms identified in these samples is given in the table on p. 129.

Report on Foraminiferal content, by W. A. Macfadyen.

S.S. (1). Fine grey clay, with traces of peaty matter. Residue large, nearly all of peaty fragments. Foraminifera very few. Brackish, approaching fresh water.

S.S. (2). Fine grey clay, with traces of peaty matter. Residue medium-sized, nearly all of peaty fragments. Foraminifera fairly frequent. Brackish, approaching

fresh water; but the presence of one or two specimens of marine species suggests proximity of the sea at least, or possibly some rare and rather remote connexion with the sea.

'The smallness of the samples was not conducive to finding really representative Foraminiferal faunas, but they seem to have been adequate for a first approximation.'

A list of the Foraminifera identified in these samples is given in the table on p. 129.

The correspondence in the deductions separately made by Herr Brockmann and Dr Macfadyen is so striking that there can be little doubt of the circumstances in which this blue clay was laid down.

G. Erdtman (1928) has recorded from 'a dry bank of peat at Shapwick' the following profile:

	cm.	
A	0– 65	Slightly decayed *Sphagnum* peat.
B	65–130	Much decayed *Sphagnum* peat.
C	130–330	Forest peat with *Menyanthes* in the lower part.
D	330–850	Grey clay.

This series corresponds quite well with those we have already described, showing the twofold division of the upper peat and indicating wetter conditions below the forest peat. It has the advantage also of having proved a depth of at least 5·20 m. of the blue clay. We already know, however, from a boring made in the turf moor near Shapwick Station in 1880, that a long sequence of most interesting alluvial deposits underlies the peat. This boring gave:

(Hosier, 1880)	ft.	in.	Approx. O.D.
			-----(+4·25 m.)
Peat	16	0	+14·0 ft.
Blue clay, containing 'lime-wash'	9	10	
Clay and sand	5	0	
Sand, hard, clean and sharp, like silver sand		10	
Quick sand	14	3	
Clay containing sand	4	6	
			------−36·5 ft. (−11·2 m.)
Peat, very dense, containing sticks, leaves, etc.		4	
Sand and gravel	4	2	
Gravel containing ragged pieces of Lias		11	
Blue clay	1	6	
			------−43·4 ft. (−13·2 m.)
Peat, containing rushes and roots well preserved		3½	
Black earth containing gravel, very dense	2	0	
Gravel sharp and broken	1	7	
			------−47·3 ft. (−14·4 m.)
Shale, perfectly dry		10½	
Lias rock, penetrated for 8 ft.			

Pollen analysis of the lower peat layers (and other beds if they were suitable) would give results of great interest particularly in relation to the course of marine transgression and retrogression which has already been traced at Swansea on the other side of the Bristol Channel (Godwin, 1940*a*).

(*c*) *Meare Heath I*

In July 1936 a peat cutter on Meare Heath came upon some sherds of a vessel of Neolithic 'B' (Peterborough) type. Their provenance was most carefully recorded by Mr H. St George Gray. They were 'about 135 yd. south of "Heath Rhyne", and 500 yd. east of the road connecting Ashcott and Meare Railway Station with the village of Meare', and '765 yd. south-west of Stileway Farm and 1160 yd. north-east of the Railway Station'. The broken pot was 4·75 ft. (1·42 m.) below the peat surface, and it was clear that this depth was correct to within 2 or 3 in. When the site was visited for the collection of pollen samples the lateral position was re-established within a few feet, but the vertical position of the pot was probably fixed to within 6 or 9 in. (15–22 cm.) either way.

Meare Heath is again a raised-bog, and the site of the discovery lay within 100 yd. or so of the bog margin. At the site all the fresh upper *Sphagnum* peat had just been removed to facilitate cutting of the dark lower peat, but along the same cutting a few yards away the Boundary Horizon was quite clear at the top of the cutting, confirming the view based on direct inspection of the disturbed surface peat at the site, that the present ground surface was about 20 or 30 cm. over the Boundary Horizon. The recorded profile is:

cm.	
0– 40	Crumbly peat soil
40–170	Dark, highly humified *Sphagnum-Eriophorum-Calluna* peat.
170–300	Black reed or *Phragmites* peat, full of fine rootlets, occasional twigs and seeds of *Menyanthes*.
300	Soft blue clay.

In a cutting at the site it was possible to make out at the top:

cm.	
0– 25	Crumbly peat soil.
25– 30	Fairly fresh *Sphagnum* peat.
30	Chocolate brown *Sphagnum-Calluna-Eriophorum* peat.

It is clear from these sections that the Neolithic 'B' pot must have been found in the lower part of the old *Sphagnum-Eriophorum-Calluna* peat, i.e. more than a metre below the Boundary Horizon, and that nevertheless it was deposited a good deal after the blue clay formation had ended.

(*d*) *Meare Heath II*

In June 1937 an attempt was made to collect material from the site where a late Iron Age (La Tène II) scabbard of bronze had been discovered (St George Gray, 1929, 1930).

The site was roughly indicated by a peat digger, Mr P. Mullins, who explained that all peat from the immediate neighbourhood had since been cut, and our own boring was made from a peat bank 10 yd. east of the stated site. Even here some of the surface peat had been removed. According to the finder the scabbard had been

found at a depth of about 2 ft. 9 in. (84 cm.). It was possible to examine the top metre of peat in a profile freshly cut for the purpose: but even so it presented a very baffling appearance. The recorded section is given below:

cm.	
0– 20	Much compressed dark brown *Eriophorum vaginatum* peat penetrated by living rhizomes of *Pteridium*. *Rhyncospora alba* rhizomes doubtfully present.
20– 36	Yellowish black peat with abundant *Phragmites*, *Menyanthes* seeds occasional 'hypnum' moss and *Eriophorum angustifolium* rhizomes.
36– 40	Extremely abundant *Phragmites* leaves and rootlets and abundant twigs. Abundant moss capsules.
40– 60	Reed-swamp peat with abundant twigs and leaves of *Myrica* or *Salix* at top.
60–100	Grey black very humified peat with abundant fine rootlets, penetrated thickly with vertical reed stems. Possibly a decayed *Sphagnum* peat with secondary penetration by *Phragmites*.
100–103	Occasional *Eriophorum vaginatum*.
103–140	Yellow reed-swamp peat (H = 3), R = 3, twigs occasional.
140–200	Highly humified *Eriophorum vaginatum* peat (H = 8–9), W = 0–1.
200–300	Highly humified reed-swamp peat (H = 9), R = 2, *Menyanthes* occasional.
300–325	Soft grey clay.

The upper peat contains a remarkable admixture of eutrophic and oligotrophic species, recalling the character of drainage areas on the surface of some raised-bogs or at their margins, but we should need extensive borings to explain it fully, and very little now remains of the upper peat hereabouts. Under these conditions we need not expect to find the Boundary Horizon.

The uncertainty as to the depth of peat removed makes it impossible to re-establish the horizon of the scabbard satisfactorily. If one spit of turf only has gone it was 54 cm. below the top of our series, if two then about 24 cm. below its top. Such suggestions are further weakened by recognizing that the bronze may have sunk from its original level, or may have been buried from the contemporary surface. All we can say is that our levels of 54 or 24 cm. are not likely to be *younger* than La Tène II: they may easily be rather older.

(*e*) *Meare Lake Village*

To the north of Shapwick Heath and Meare Heath is the low ridge of upland which carries the villages of Meare and Westhay. Beyond this ridge is a separate system of fens and bogs traversed by the artificial courses of the Brue and its many large tributaries. These peat lands are now mostly pasture or arable ground, and it is in pastures on the north flank of the ridge that Dr Bulleid and Mr St George Gray have excavated two considerable Iron Age villages. It appears that they were

occupied from not much later, if any later, than 300 B.C., until well on into the Roman period. At the eastern of the two villages we were allowed by the courtesy of the investigators to make observations of the stratigraphy and to take samples for pollen analysis. Very low circular mounds in the pasture betray the floors and hearth sites of the village huts, raised by successive layers of ash and of clay (Fig. 3). A layer of grey clay or of black soil extends from one hearth to another and this has been clearly established as the occupation layer. Both it and the hearths are covered by 60–80 cm. of buff clay, presumably a later deposit due to river flooding. A site for record and sampling was chosen 15 ft. (4·6 m.) west-north-west from the central picket of mound XXI and 27 ft. (8·2 m.) east of the centre picket of mound

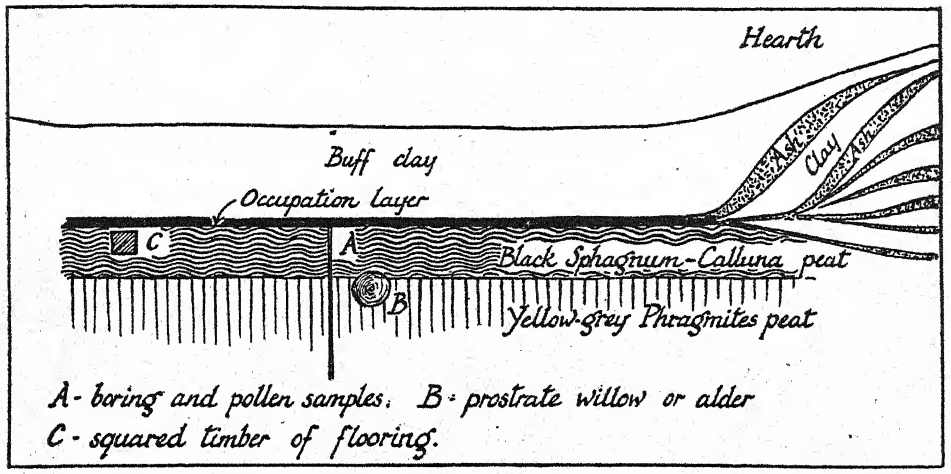

Fig. 3. Diagrammatic profile to show the stratigraphy exposed to direct examination at the site in the east Lake Village at Meare.

XV B. A clear profile was visible to 1·40 m., and below this the borer was used. The gross section is as follows:

cm.	
0	Grass surface.
0– 60	Buff clay.
60– 67	Grey clay with organic content (occupation layer).
67– 80	Crumbly, black, amorphous *Sphagnum-Calluna* peat.
80– 85	Very black *Calluna* peat, *Calluna* very abundant.
85– 90	Black amorphous peat with less *Calluna* and more twigs.
90–120	Very compressed and fresh yellow-grey *Phragmites* peat with large horizontal stem of *Alnus* or *Salix* at about 95 cm. and wood rather frequent throughout.
120–200	Brown amorphous lake peat.
200–275	Wood peat—transitional to lake detritus peat in the upper part.
275–300	Stiff blue clay.

About 7 yd. west of A the details of the upper layers were somewhat clearer:

cm.	
0– 81	Buff clay.
81– 92	Crumbly, granular brownish black peat.
92–106	Raised-bog peat with *Sphagnum* band at 92, *Eriophorum vaginatum* at 99, and *Calluna* at 105.
107	*Molinia* or sedge.
107–120	Black peat with *Phragmites* and frequent small wood.
120	Transition to yellow compact *Phragmites* peat.

In the excavated material the compressed and oxidized, but quite recognizable pieces of *Sphagnum-Calluna-Eriophorum vaginatum* peat were found, so that it was clear that the village had been built upon a raised-bog, or at least the marginal part of such a bog. This fact, not previously recognized, goes far to explain the substantial difference in construction of the Meare village from that at Glastonbury. The latter was built upon a thin layer of wood peat which overlaid deep lake-detritus peat. The horizontal layers of beams and brushwood of the flooring were tied and supported by vast numbers of more or less vertical stakes driven deep into the soft peat below. At Meare such piling or staking is practically absent, and the settlement rested upon a rather sparse arrangement of horizontal timbering and in places had none at all. This would be quite a feasible arrangement on the margin of a raised-bog, the oligotrophic character of which itself indicates immunity from flooding. Added firmness would be given to the site by the rather frequent tree-remains in the *Phragmites* peat below the *Sphagnum* peat. 'Lake Village' is indeed perhaps a less suitable term for the settlement here than for that at Glastonbury.

A very close idea of the stratigraphy throughout the Meare section can be obtained from the careful analysis made by Dr M. H. Clifford of the larger detritus in the pollen preparations (see table on p. 124).

As one would expect, both the tree pollen and non-tree pollen counts from samples at this site are much affected by the local conditions, and strongly reflect the formation of wood peat and of raised-bog at appropriate horizons.

POLLEN ANALYSES

A consideration of the pollen analyses from the Somerset sites involves:

(1) The recognition and discounting of the local influence of trees growing actually upon the peat surface.

(2) The co-ordination of the Somerset sites with one another on the basis of the general forest composition of the countryside as a whole.

(3) Correlation of the Somerset pollen series with similar series from other parts of the country.

(4) Consideration of the way in which these correlations correspond with archaeological, geological and climatic indices.

(*a*) *Local comparisons*

If we compare the three pollen diagrams, Shapwick Heath, and Meare Heath I and II, a very great similarity will be observed between them, more particularly at the bottom. In each the same sequence will be observed above the blue clay, and within the bottom 1·0–1·2 m. of reed peat *Ulmus* is maintained at strikingly high values, and *Tilia* behaves similarly although present in smaller absolute amounts. At the end of the reed-swamp phase there is at Shapwick a pronounced wood-peat horizon, and this is clearly reflected in the pollen diagram as a big peak in the *Betula* pollen. Similar but smaller peaks in the two diagrams for Meare Heath doubtless also reflect the same local development of birch woods on the reed peat, and we may note that smaller maxima about half-way down the reed peat correspond in the three diagrams (Figs. 5–7) (possibly with *Pinus* at Shapwick) and may represent the lower wood layer, indications of which were mentioned in the Shapwick profile (p. 113). We may note that *Pinus* is more strongly represented in the reed peat of all the diagrams than above the big *Betula* maximum which marks the transition to *Sphagnum-Calluna* peat. Above the *Betula* maximum the three diagrams maintain a close correspondence. *Alnus* pollen now rises to considerable importance, and although this might be explicable by the extensive development of alder woods round the margins of the developing raised-bogs, it seems remarkable that the earlier wood-peat horizons did not produce similar effects by invasions of alder upon the reed-fen surfaces. All three diagrams at this level remain closely similar, and it will be particularly noted that *Ulmus* and *Tilia* values remain high though less than in the preceding period. With the onset of ombrogenous peat formation above the *Betula* maximum the *Corylus* curve rises very abruptly in all three diagrams, but the author is much inclined to question the validity of this as a true indication of hazel. Throughout the Meare and Shapwick Heath *Sphagnum* peats, the spiky twigs (and sometimes leaves) of *Myrica gale* are present, and pollen referable to this plant is present in such amount as often to outnumber the total tree pollen many times. Although every effort was made to distinguish *Corylus* from *Myrica* pollen, there remained a proportion of grains very difficult to refer accurately to one or the other type. These results very strongly suggest that much of the 'Corylus' pollen in the *Sphagnum* peats ought to have been identified as that of *Myrica*. It is fortunate that few British bogs have such overpowering local concentrations of *Myrica*, that these effects are present. In small amount doubtless *Myrica* can be effectively separated from *Corylus*: here the *Corylus* curve is almost entirely invalidated.

In the Meare Heath II diagram it is apparent that a general change has set in at the 55 cm. level. A small *Pinus* maximum is followed by a steady and big rise of *Betula*, and a small *Ulmus* maximum, the *Tilia* curve ceases and to some extent *Quercus* replaces *Alnus* as the dominant tree pollen. A precisely similar behaviour can be seen in the Shapwick Heath diagram where the *Pinus* maximum is at 205 cm. A short distance above the *Ulmus* maximum falls the Boundary Horizon and *Fagus* pollen is present almost continuously from this level upwards. It is apparent that,

as the field work showed, much of the upper peat is missing from Meare Heath II, and it seems that, roughly speaking, everything down to the Boundary Horizon has disappeared. In Meare Heath I the characteristic features of this third phase of the pollen diagrams are absent, save for termination of the *Tilia* curve, and it is apparent

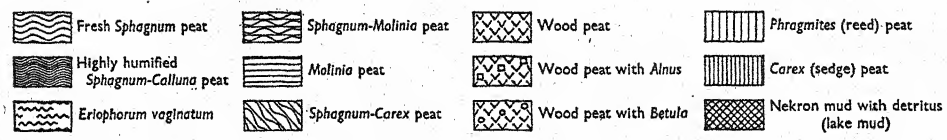

Fig. 4. Key to the symbols employed in the pollen diagrams of Figs. 5, 6, 7 and 8 for the different peat types recognized.

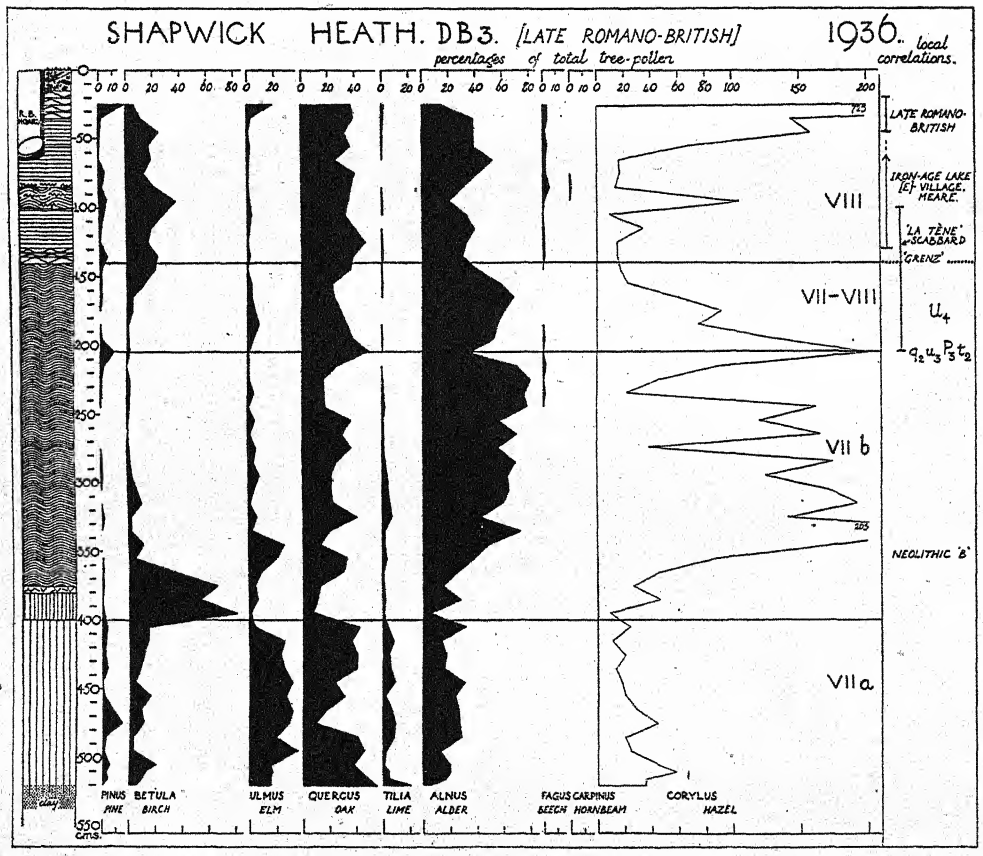

Fig. 5. Pollen diagram on Shapwick Heath, Somerset, at the boring adjacent to the late Romano-British hoards. The presumed levels of other adjacent bog discoveries have been transferred to this figure on a basis of the sum of the pollen curves. Certain of the characteristic maxima and minima are lettered as by Hardy (1939) for Shropshire pollen diagrams. The pollen zoning shown by Roman letters is that used by the author for England and Wales as a whole (Godwin, 1940*b*). Peat symbols as in Fig. 4.

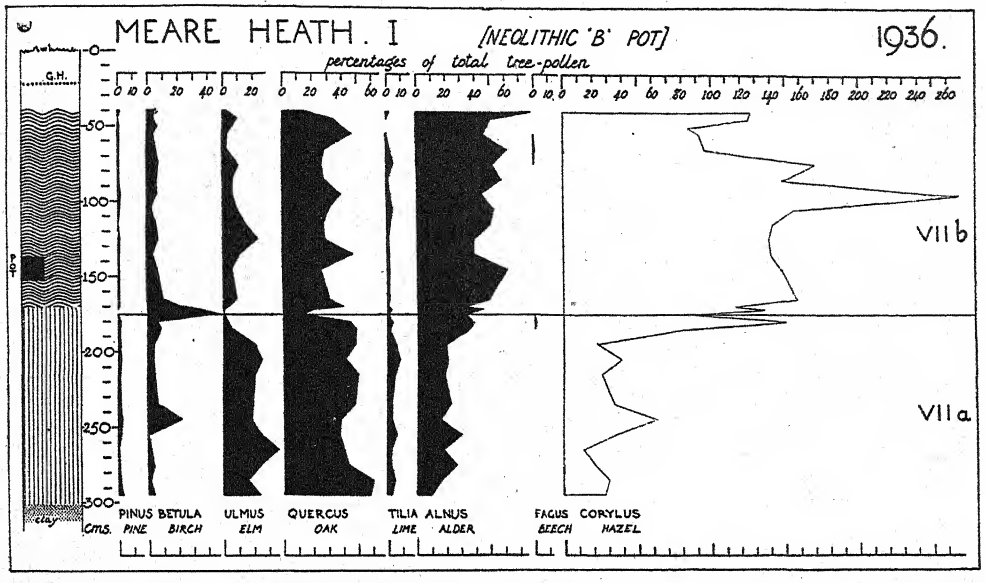

Fig. 6. Pollen analysis on Meare Heath, Somerset, within a few feet of the site from which sherds of a Neolithic 'B' pot had been taken. The upper *Sphagnum* peat had been removed down to the Boundary Horizon (G.H.). Peat symbols as in Fig. 4.

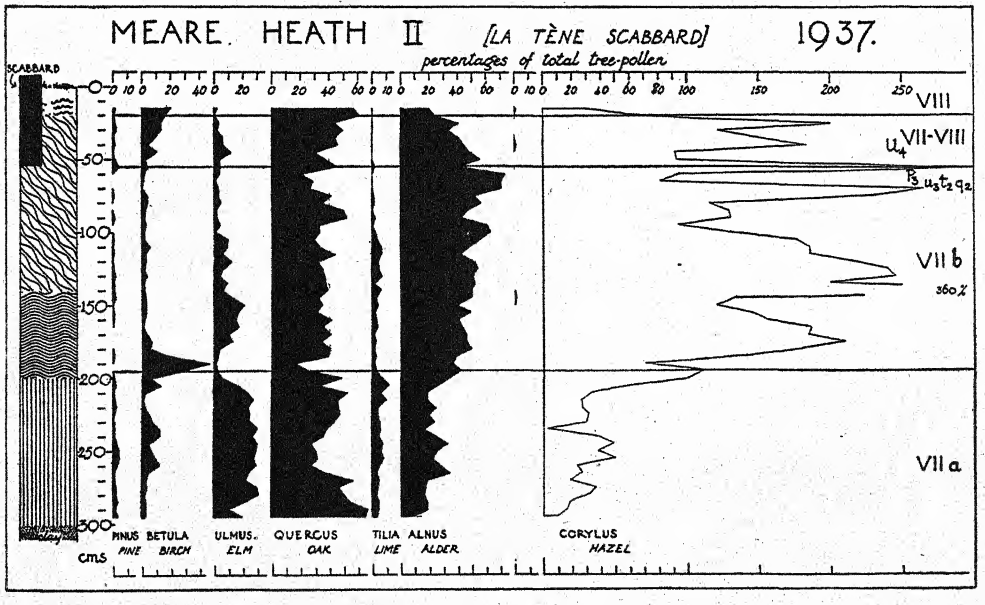

Fig. 7. Pollen analysis on Meare Heath, Somerset, close to the site at which a La Tène bronze scabbard had been discovered. The scabbard is unlikely to have come from a level below 54 cm. under the present surface. Peat symbols as in Fig. 4.

Table 1. *East Lake Village—Meare*

Peat type	Depth from surface cm.	*Typha* sp.	*Phragmites communis*	*Carex* spp.	*Alnus glutinosa*	*Betula* sp.	*Salix? cinerea*	*Quercus* sp.	Fern sporangia	Sphagna	Remarks
Occupation layer	65	—	—	—	—	—	—	—	—	—	Silt and coarse sand, occasional charred wood fragments, fruit of *Sambucus nigra*
	70	—	—	—	—	—	—	—	—	—	Silt and two fragments of charred wood
Raised-bog *Sphagnum* peat	75	—	—	—	—	—	—	—	—	—	Chiefly *S. cymbifolium*, some *S. acutifolium*. Leaves of *Eriophorum* sp.
	80	—	—	—	—	—	—	—	—	ab.+	Exclusively well preserved Sphagna. *S. acutifolia* and *S. cuspidata* groups
Wood peat	85	—	—	rare rtls.	—	fr.	—	—	ab.	+	Transition peat. Sphagna occasional but eroded. Rare small wood and leaf fragments. ?*Drepanium* sp.
Carex and *Typha* peat	90	fr.	—	ab. rtls. frs.	—	—	—	—	frq.	—	*Carex* peat with rootlets abundant. Frequent fruits of *Hydrocotyle vulgaris*
	95	frs. v. ab.	rtls.	rare rtls.	—	—	—	—	—	—	Fibrous rootlet peat
Woody reed-swamp peat	100	—	—	—	? —	lvs.	—	—	—	—	Wood peat, ? some traces of charcoal
	105	—	—	—		—	—	—	+	—	Rhizome and rootlet debris
	110	—	—	frs.	—	tw.	lf.?	—	—	? root	Wood peat with some rhizomes. Rare Sphagna
	115	frs.	rtls.	rtls.	—	—	—	—	—	—	A rootlet peat with traces of wood and some sedge and reed. Fruits of *Juncus glaucus-effusus* group
	120	—	—	—	—	—	—	—	—	—	?A gramineous peat with some *Carex* rootlets
Detritus nekron mud	125	—	—	v. rare rtls.	—	—	—	—	—	—	?Aquatic rootlet peat
	130	—	—	rtls. fr.	—	—	—	—	—	—	Very fibrous *Carex* rootlet peat
	135	—	—	frq. rtls.	?	?	—	—	frq.	—	
	145	—	rtls.	v. rare rtls.	—	—	—	—	—	—	
	155	—	rtls.	rtls. fr.	—	—	—	—	—	—	*Phragmites*-Cyperaceae peat with one piece of very decayed wood
	165	—	—	rare rtls. frs.	—	—	—	—	—	—	Rootlet peat with rare to occasional wood
	175	—	—	—	tw.?	tw.?	—	—	—	—	Rootlet peat with rare to occasional wood
	185	—	ab. rtls.	ab. rtls.	tw.?	tw.? ? fr.	—	—	—	—	Wood fragments with abundant *Phragmites* and *Carex* rootlets
	195	—	—	—	—	—	—	—	—	—	Abundant fruits of *Eupatorium cannabinum*, a few wood fragments, abundant rootlets
Wood peat	205	—	—	—	? wd.	—	—	—	—	—	Definitely a wood peat
	215	—	—	—	—	fr.	—	—	+	—	Eroded leaves of *Thuidium*? *tamariscinum*, bulky wood fragments
	225	—	—	—	frs. ? wd.	—	—	lvs.	rhz.+	—	Wood peat, of alder (?). Some fern wood tissue
	235	—	—	—	fr. cone scales	—	—	lf.	—	—	Wood peat
	245	—	—	—	frs. st. tws.	—	—	lf.	—	—	Wood peat. Alder from stem twigs. ?*Hylocomium* sp.
	255	—	—	—	—	—	—	—	+	—	Wood peat
	265	—	—	—	ab. frs.	—	—	lf.	—	—	Wood peat
	275	—	—	—	ab. tws.	—	—	—	—	—	Wood peat, alder twigs abundant, some rootlets
	280	—	—	—	—	—	—	—	—	—	Very silty wood peat

KEY

fr. (frs.) = fruit(s) or seed(s) tw. (tws.) = twig(s) st. = stem wd. = wood lf. (lvs.) = leaf (leaves)
rhz. = rhizome rtls. = rootlets v. = very frq. = frequent ab. = abundant

that here even more peat has been removed. This corresponds with the position shown by the field observations for the position of the Boundary Horizon some 20 cm. above the uppermost analysed sample.

The close correspondence of the three diagrams makes practically certain the fact that the blue clay surface is of the same age in each, that wood peat and ombrogenous peat formation began at the same time in all three, and that the Boundary Horizon formed at the same time in Meare Heath II and on Shapwick Heath. The uniformity of the pollen curves allows us to transfer to the Shapwick diagram the archaeological horizons of the two Meare Heath sites: the Neolithic 'B' horizon can be put at about 350 cm. on it, and the La Tène level would lie between about 205 and 100 cm.

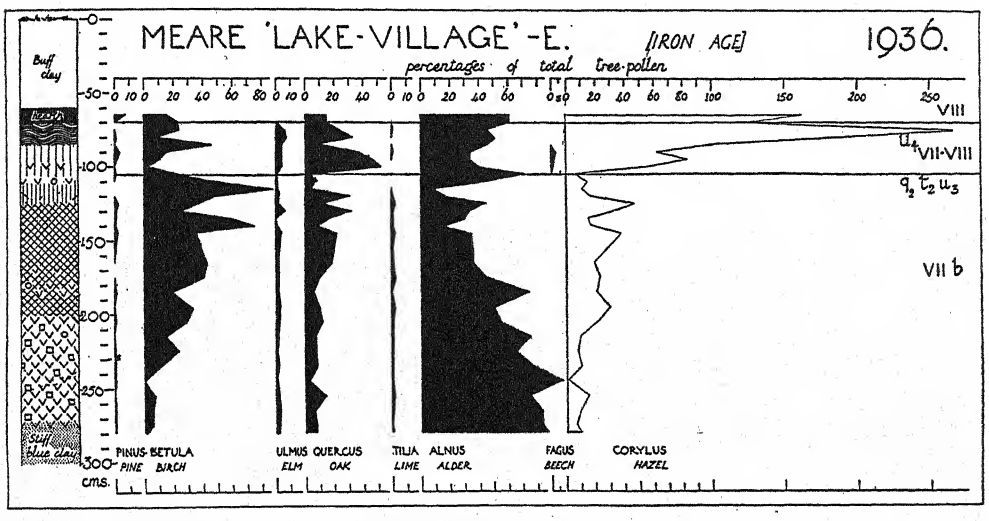

Fig. 8. Pollen diagram from samples beneath the occupation layer at the east Lake Village, Meare. Peat symbols as in Fig. 4. The abundant wood in the peat profile is reflected in large amounts of alder and birch pollen in the diagram.

In order to be able to insert more accurately into the Shapwick diagram the late Romano-British horizon, when the second hoard was found samples were carefully collected from the grass-like wrapping surrounding the small clay vessel which contained the coins, and from material pushed into the pot. The pollen analyses of these samples are given below:

Wrapping of Roman pot at Shapwick Heath (1937)

	Percentage of total tree pollen							
	Pinus pine	*Betula* birch	*Ulmus* elm	*Quercus* oak	*Tilia* lime	*Alnus* alder	*Fagus* beech	*Corylus* hazel
Over the pot	0	13·5	0	55	+	31·5	0	156
Round the pot	1	8·5	1	67·5	1	20·5	0	335
Inside the pot	2·5	9·5	1·5	53	1·5	31	+	515

The *Pinus*, *Betula*, *Ulmus*, *Tilia* and *Fagus* values agree well enough with levels 35–65 cm.—(i.e. not far above the level of the hoard itself). The *Quercus* is higher, and the *Alnus* lower than in the Shapwick figure at these levels, but such a discrepancy might be expected in packing material evidently not picked from the bog itself.

It will now be possible to consider the diagram from the Meare Lake Village site as it appears in relation to the Meare Heath and Shapwick series. It has been already demonstrated that much of this peat is itself wood peat or contains abundant wood, and there is little doubt that the high values for *Betula* from 100 to 230 cm. in the Lake Village diagram (Fig. 8) are due to the local influences of fen woods or fen scrub. Similarly the high *Alnus* values in the bottom 50 cm. of peat must be discounted as a local effect. If, with this in mind, we pay attention to the other tree-pollen curves it will be seen that at 105 cm., *Tilia* virtually ceases, and just above there is a good *Ulmus* maximum, some *Fagus*, and high values of *Betula* not likely to be due to local influences. It appears in short, that this level marks the onset of the third of the phases into which were divided the diagrams already considered. The *Pinus* values do not contradict this view, although not in themselves of much indicator value. The Lake Village horizon may therefore, by means of the pollen curves, also be transferred to the long Shapwick diagram, with the recognition that possibly some removal by oxidation from the *Sphagnum-Calluna* peat should be allowed for. It is not easy to compare the base of the Lake Village series with the other diagrams, but it is evidently younger at the base, so that wood-peat formation on the flanks of the hard Lias clay island here began *after* the reed peat had started to form over the Shapwick and Meare Heath region.

Combwich.

On the estuary of the River Parrett, four miles north-west of Bridgwater, and beside the village of Combwich, is a deep brick-pit. The ground level is here about 20 ft. above mean sea-level, and on the surface Mr H. S. L. Dewar (1941) has reported to us the finding of abundant Romano-British potsherds.

At the brick-pit we determined the stratigraphy shown in Fig. 9. Below the upper clay, which is about 14 ft. thick, the digging had exposed an extensive peat layer about 8 in. (20 cm.) in thickness, and containing the stumps and prostrate trunks of scattered small trees. These were identified as mostly alder (*Alnus*), although several yews (*Taxus*) were present. Below this, peat boring showed that there was 6 ft. (1·83 m.) of blue clay, and then a black reedy layer of fen peat with a high clay content and only one or two centimetres in thickness. This overlaid more of the blue clay. The beds were related to Ordnance Datum by levelling from a spot-level in the village.

One large sample of the upper clay was taken at 1·40 m. below the surface (Cb. (1)) and a smaller sample of the clay between the two peat-beds (Cb. (2)) at 4·80 m. below the ground surface (see Fig. 9). The faunal content of these samples were reported upon by Dr Macfadyen and Herr Brockmann as follows:

Report on Foraminifera, by Dr W. A. Macfadyen.

Cb (1). Fine grey clay mottled with light brown, many fine holes as of vanished rootlets. Residue very small, fragments of hardened silty material, sometimes iron-stained, a few quartz grains, and a few rootlets. Foraminifera nil.

This suggests fresh-water conditions.

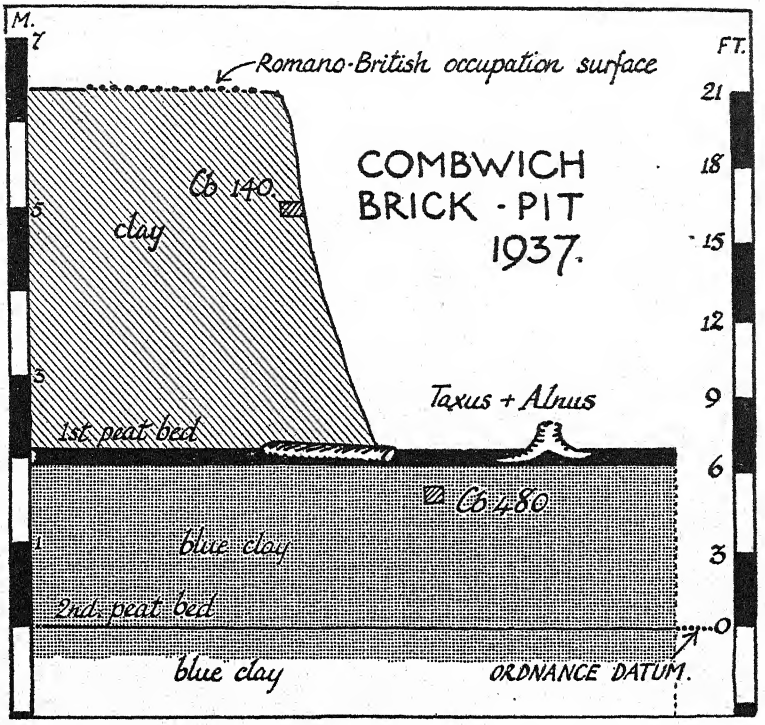

Fig. 9. Diagrammatic profile at the brick-pit at Combwich, Somerset, on the banks of the River Parrett. The sites are shown from which samples were taken for microfaunal analysis (Cb (1) at 140 cm. and Cb (2) at 480 cm.).

Cb (2). Mottled grey and light brown fine clay, with many rootlets. Residue very small, largely rootlets, with a minute amount of inorganic material. Foraminifera frequent (see Table 2, p. 129).

This suggests brackish water conditions of deposition, somewhat more marine than those for S.S. (2) (the deeper clay sample at Shapwick Station).

Report on Diatoms, by Chr. Brockmann.

Cb (1). *Ziegeleiton.* 'Keine Diatomeen gefunden, wenig Kieselnadeln von Schwämmen.'

Although no diatoms were found 'damit ist noch nicht gesagt, das sie *nicht* unter Einwirkung des Meerwassers abgelagert sind. Bei genügender Ausdauer würde man wahrscheinlich auch in den "leeren" Proben einige Schalen finden.'

Cb (2). *Ziegeleiton.* 'Sehr wenig Diatomeen, nur marine Formen', 'der Probe ist ein deutlicher brackischer Einschlag festzustellen, sodass diese Ablagerung sicher als brackischmarin zu bestimmen ist.'

There is thus little evidence directly in favour of the upper brick clay being brackish or marine, but in view of its position and level it would be unlikely to be entirely fresh water in origin. The clay below the upper peat was quite clearly deposited under rather strongly marine conditions. As its surface is now about +6·5 ft. O.D., the possibility arises at once that it is part of the same phase of marine transgression as the clay, the surface of which lies about +6·0 ft. O.D. under Shapwick Heath, and at about +4·0 ft. O.D. at Shapwick Station. It is naturally somewhat more marine in character at the seaward site.

Pollen analysis of the upper peat layer cast a little light upon this correlation. Four samples were taken at short intervals through the bed, but they proved difficult to analyse on account of their clay content and sparseness in pollen.

Percentage of total tree pollen

Cm. below surface of peat layer	*Pinus* pine	*Betula* birch	*Ulmus* elm	*Quercus* oak	*Tilia* lime	*Alnus* alder	*Corylus* hazel
3	1·5	2	11	54	4·5	27·5	51
7	2	1·5	7	70	3	16	25·5
11	+	2	11	57	+	28·5	50
15	1	2	7·5	62	2·5	25·5	90

From this table the high *Ulmus*, substantial *Tilia*, and low *Betula* values stand out clearly, and suggest equivalence with the lowest or middle sections of the Meare Heath and Shapwick Heath diagrams. That is to say, this Combwich peat predates the Boundary Horizon. More exact dating of these coastal peats must await much wider stratigraphic as well as pollen-analytic study.

(*b*) *General comparisons*

When we consider how these Somerset results fit into the wider picture of post-glacial evolution of this country several features of interest become evident. It is clear that the pollen series now produced begin when the alder is already an important component of the woodlands; the reed peat is referable to the wide zone VII (alder-oak-elm-lime zone) of our scheme of forest history (Godwin, 1940*b*), and must be post-Boreal in age.

The Somerset series are remarkable in that they have at the base maintained values of 20–30% of *Ulmus* pollen. Such large amounts have not hitherto been recorded elsewhere in Britain, nor is it apparent how the conditions of time and place were responsible for them.[1] Hyde (1940) suggested a division of zone VII into two subzones, of which the upper (*a*) was separated from the lower (*b*), by a

[1] In reply to my recent enquiry of him upon this point Mr Melville of Kew kindly writes: 'Presumably... the springs were warm and moist, and late frosts rare once the winter had broken... just the conditions in fact to favour the fruiting of elms', and 'observations in the field go to indicate that the elms as a group are lovers of moist conditions'.

definite and lasting decrease in *Ulmus* pollen, and a similar decrease in the *Pinus* and *Betula* percentages. This is clearly brought out in the diagram from Ffos-ton-Cenglau, Glam. where the *Ulmus* has generally high values, and it can be recognized

Table 2. *Lists of Microfauna in clays from the Somerset Levels*

Diatoms, identified by Chr. Brockmann	Shapwick Station		Combwick Brick Pit		
	380–400 cm. S.S. (1)	440–500 cm. S.S. (2)	140 cm. Cb (1)	480 cm. Cb (2)	
Melosira westi	+	+	.	r.	
M. sulcata	r.	+	.	r.	
Podosira stelliger	+	+	.	r.	
Cyclotella striata	.	+	.	.	Brackwasser
Coscinodiscus radiatus	.	+	.	.	
Actinoptychus undulatus	.	r.	.	.	
Triceratium favus	r.	r.	.	.	
Raphoneis surirella	.	r.	.	.	
Scoliopleura tumida	.	r.	.	.	
Diploneis didyma	.	+	.	.	Brackischmarin
Nitschia punctata	r.	r.	.	.	
N. navicularis	.	+	.	.	
N. constricta	.	r.	.	.	
N. vitrea	.	r.	.	.	
Foraminifera, etc., identified by W. A. Macfadyen					
Quinqueloculina subrotunda (W. & J.)	.	.	.	I	
Cornuspira involvens (Reuss)	.	.	.	I	
Trochammina inflata (Montagu)	I	V	.	.	
T. macrescens (Brady)	V	V	.	V	
Virgulina fusiformis (Williamson)	.	.	.	I	
Bolivina pseudoplicata (Heron-Allen & Earland)	.	.	.	I	
Cassidulina crassa (d'Orbigny)	.	.	.	I	
Lagena stewartii (Wright)	.	I	.	.	
Discorbis chasteri (Heron-Allen & Earland)	.	.	.	I	
Rotalia beccarii var. *lucida* (Madsen)	.	L	.	L	
Gyrodina soldani (d'Orbigny)	.	.	.	I	
Nonion depressulus (Walker & Jacob)	.	.	.	V	
N. grateloupi (d'Orbigny)?	.	.	.	I	
Elphidium excaratum (Terquem)	.	.	.	V	
Gasteropods, non-marine	.	.	.	I	
Seeds	.	.	.	X	
'Tailed brown globes'	.	.	L	C	

+ = present, r. = rare, I, V, X, L and C are a scale of increasing frequency.

at Tregaron (boring S.E. 10, Godwin & Mitchell, 1938). The widespread character of this rather small change in forest composition has already been demonstrated (Godwin, 1940*b*).

It is interesting to note that in comparison with the East Anglian Fenland *Ulmus* and *Tilia* have exchanged roles of relative importance in zone VII, *Ulmus* being more important in the West and *Tilia* in the East.

The uppermost section of the Shapwick pollen series shows big changes in forest composition, which have been useful in co-ordinating the separate Somerset sites. These changes have, however, a wider importance for they represent the phenomenon of revertence in forest history which has allowed the clear separation of zone VIII, an alder-oak-elm-birch-(beech) zone for England and Wales as a whole. *Betula* has everywhere increased, *Tilia* has virtually ceased, and in some parts of the country *Fagus* and even *Carpinus* are continuously present. It is with

Table 3. *Somerset first correlations*

Forest periods		Archaeological horizons	Breaks in peat formation	Raised-bog stratigraphy
VIII	rather high *Betula*. *Fagus* present	Romano-British hoards and occupation Iron Age	14 ft. Clay (Combwich)	? growth ceased young *Sphagnum*-*Molinia* peat
				—Boundary Horizon—
VII–VIII	U_4 rising *Betula*. P_3t_2	La Tène		old *Sphagnum*-*Calluna* peat
VII*b*	diminution	Neolithic 'B'		—Tree Layer—
VII*a*	of *Ulmus* and *Pinus*		Brackish marine clay (Shapwick)	*Phragmites* peat (Tree Layer) *Phragmites* peat

the Shropshire mosses, however, that the closest comparison is possible, where Miss Hardy (1939) recognized a transition zone, VII–VIII, and employed a special notation for distinctive maxima and minima in the pollen curves. The end of zone VII was marked by a maximum of *Pinus* (P_3) and minima of *Tilia* (t_2), *Ulmus* (u_3) and *Quercus* (q_2). Within zone VII–VIII was an *Ulmus* maximum (U_4), and the transition to zone VIII was placed where a Boundary Horizon was made by a sudden general decrease in humification of the bog peat.

These indicator points in the pollen curves are apparently recognizable in our Somerset diagrams, where they have been taken into account in deciding the zonation. It is interesting to note that in these Somerset bogs, as in Shropshire, the Boundary Horizon falls at the end of zone VII–VIII.

The conclusion of our attempted correlation between the various aspects of post-glacial evolution in this part of Somerset can be summarized in Table 3.

These conclusions seem well established and promise considerable reward to an extension of these methods of investigation in the Somerset levels. It had been hoped to push forward such investigations at once, but war-time conditions are so much against intensive field work that it seems preferable to publish what has been done as a mere account of progress. We may mention the prehistoric trackways described by Bulleid (1933), and the monoxylous boat found at Shapwick Station and recorded by the same author (Bulleid, 1906), as instances in which it now appears that pollen analysis gives promise of providing some kind of dating.

These Somerset results have already been shown to fit into the general pattern of forest history in England and Wales, and there seems every liklihood that the sensitive and characteristic pollen curves of this region could be employed to attack the important geological problem of the course of land and sea-level movement on this coast. In particular we may note that whereas in the East Anglian region the marine transgression which brought the deposition of clay farthest inland took place in late Neolithic or early Bronze Age times, in the Somerset Levels the data (though sparse) suggest that the maximum marine transgression had passed long before the Neolithic period. This indicates how careful examination of the Somerset area, in comparison with the Fenland might yield clear evidence for an isostatic component in the post-glacial land and sea-level movements of the southern half of Britain.

Summary

This paper reports the outcome of preliminary investigations of the post-glacial deposits of the Somerset levels. Shapwick and Meare Heaths are shown to be relict raised-bogs developed over a semi-marine clay, the surface of which now lies at approximately +5 ft. O.D. The ombrogenous peat of these bogs shows a strong division into a lower well-humified *Sphagnum-Calluna-Eriophorum* peat and an upper fresh *Sphagnum-Molinia* peat. A series of archaeological discoveries in the peat of these heaths, allows a rough dating of this Boundary Horizon and establishes a correlation with pollen diagrams made at each discovery site. Peat formation in these mires began in zone VII*a* (the alder-mixed-oak forest stage) including remarkably high values for elm pollen, and continued until late Roman times. When the upper layers are still present, as at Shapwick, they show the pollen composition typical of zone VIII, in which revertence in forest history is indicated by increase in *Betula* and virtual disappearance of *Tilia*: a small amount of *Fagus* is also present.

The east Lake Village at Meare has been found to rest on a thin layer of raised-bog peat, and it seems certain that the occupation surface falls near the opening of zone VIII.

Pollen and faunal analyses are reported from Combwich on the Parrett estuary, where a thick clay layer overlies a tree layer referable to zone VII.

The pollen diagrams reveal a drift of forest history quite conformable with that for the country as a whole, and the stratigraphic data reinforce the view that the structure and development of the region are very similar in principle to those recognized in the East Anglian Fenland.

ACKNOWLEDGEMENTS

In conclusion the author acknowledges not only the co-operative services so kindly offered him by Dr Bulleid, Mr St George Gray and Mr H. S. L. Dewar, but also those of the peat cutters, Mr Mullins and others who allowed access to their diggings and to their extensive experience of the turf moors. Prof. J. S. Turner helped greatly with the strenuous field work. The pollen counting was made possible by the payment of a grant from the Department of Scientific and Industrial Research, and the borings were made by a Hiller type peat drill purchased by a grant from the Royal Society of London.

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REGENERATION OF LEAFLETS IN LUPINS

By MARY SNOW
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(With 6 figures in the text)

ALTHOUGH the young leaf-tips of various ferns are known to regenerate well, yet amongst seed plants apparently the only leaves which have been shown convincingly to regenerate at all completely are the first-formed few leaves of seedlings of *Cyclamen Persicum* (see Goebel, 1902, 432; 1908, 202). These leaves, which are in some ways peculiar, can regenerate new laminae from the sides of the petiole when the main lamina is removed. Recently Figdor (1926) has claimed to have caused regeneration in leaves of *Bryophyllum calycinum*, by cutting off the terminal leaflet at a comparatively late stage, when it was 5 mm. long. In two of the leaves new structures were developed which were anatomically like leaflets: but they were extremely small and their shapes were nothing like those of normal leaflets. Indeed, the photographs suggest the question whether these structures were not formed merely by the continued growth of a small part of the base of the terminal leaflet, which had not been removed by the operation.

In previous experiments on stem apices of seedlings of *Lupinus albus* we were obliged to cut off the apical parts of certain of the young leaves or leaf-primordia because they arched over and concealed the apex. These leaves were very much younger than those operated upon by Figdor, and occasionally we noticed that one of these leaves still developed its leaflets; and so we were led to make further experiments.

In *L. albus* the youngest two leaves have no leaflets. If the leaves are numbered in order, leaf 1 being the leaf that is just arising from the stem apex—that is, the youngest visible leaf—then leaf 3, or sometimes leaf 4, is the first that shows leaflets, and this leaf shows a division into three leaflets in its apical part. Leaf 5 has added another two leaflets below these (Fig. 1 *b*, *c*), and leaf 6 another two below these again, making seven in all (Fig. 1 *a*, *d*). This at least is the most usual time-table, though it sometimes varies a little: but the sequence of formation of leaflets is always towards the base. Seven is much the commonest final number of leaflets in these seedlings, but occasionally leaves are found with eight leaflets or nine. In describing the experiments it will be convenient, as before, to call the youngest leaf that was just visible at the time of operation P_1, and the successively older leaves P_2, P_3 and so on.

The operation was usually to cut off the apical part of P_3, P_4, P_5 or P_6, together with all the leaflets present, by an approximately horizontal cut through the young leaf, which at these stages stands erect. The position of such a cut on a P_5 leaf is shown in Fig. 1 *b* by the line *xx'*. Sometimes instead the leaflets were cut off with

two sloping cuts just below their bases in the positions of the lines *yy'* and *zz'*, and sometimes they were cut or snapped off separately at their insertions: this last operation could not be done on leaves younger than P_6. In each apical bud one young leaf was split in a radial vertical plane, and by counting from this leaf the other leaves operated upon were identified subsequently. The operations were made under a binocular microscope, and after times varying from 9 to 16 days, the apical buds were pickled, and subsequently examined in transverse sections, the methods used being the same as before (Snow & Snow, 1937). For comparison successive transverse sections through and just below the bases of the leaflets of a normal leaf are shown in Fig. 2*a–d*.

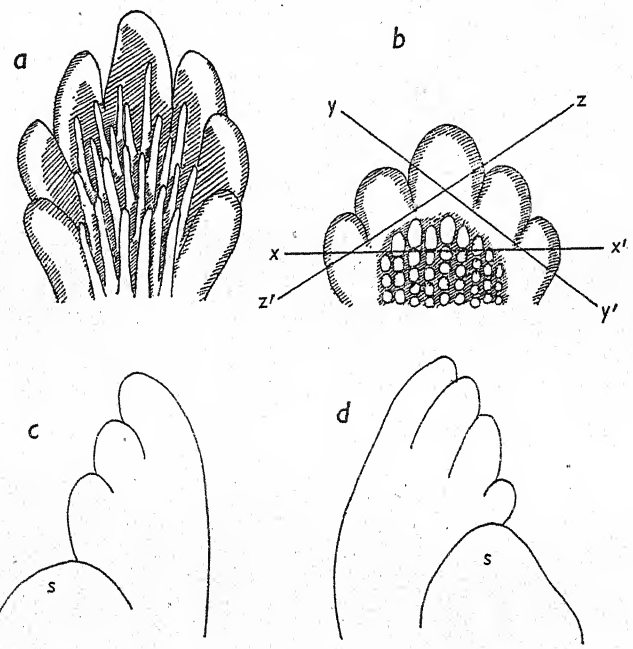

Fig. 1. Leaf-primordia cut off at base and drawn free-hand as seen in the solid: *b* and *c* are the fifth leaf-primordia, and *a* and *d* the sixth: *a* and *b* from inner (adaxial) face, *c* and *d* from one side. In *a* and *b* hairs are shown on the surface, and the stipules are omitted. In *c* and *d*, *s* = stipule.

Although about 150 leaves were operated on, usually two or three in each apical bud, only eight can be said with certainty to have regenerated leaflets. One leaf which regenerated well is illustrated in transverse sections from above downwards in Fig. 3*a–f*. This leaf, no. 1, was operated upon at the P_5 stage, and had its apical part, including all the five leaflets present, removed with a horizontal cut. After 12 days it was found to have seven leaflets, arranged irregularly.

It may be suggested that some of these leaflets were not regenerated, but were merely those normal leaflets which had not yet arisen at the time of operation. This suggestion could not in any case apply to more than four of the seven leaflets found, since five had been removed, and a total of more than nine was never found in

normal leaves, while a total of seven was much more common. But further, since normal leaflets are formed in basiscopic sequence, it would hardly be possible for more than two such leaflets, the two adjacent to the wound, to show wound scars: yet as the drawings show, five of the seven leaflets found had wound scars on their backs or sides. It can also be seen that the highest leaflets were not formed in basiscopic sequence at all, but practically at the same level. So it seems certain that at least four of the leaflets were formed by regeneration.

The drawings also indicate that several of the leaflets were formed from the dorsal (adaxial) face of the leaf, and not from its edges like normal leaflets. This is clear from their positions relative to the shaded moribund piece of tissue, which was the most apical part of the leaf that remained after the operation. This part is dying presumably because it no longer has any leaflets inserted above it.

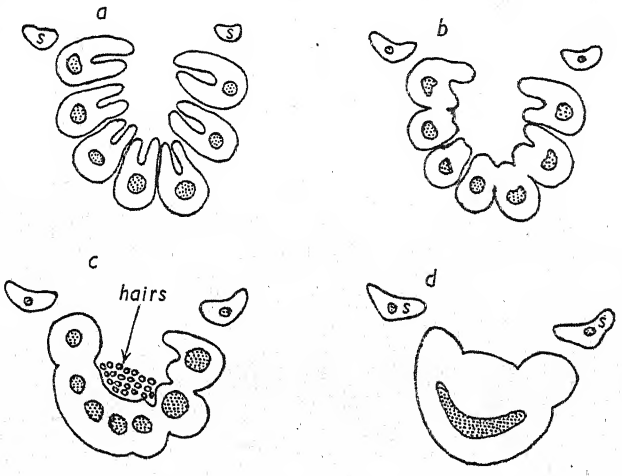

Fig. 2. Successive transverse sections through a normal young leaf, with seven pinnae and two stipules. Conducting tissue is dotted. *s*=stipule. ×39.

It may be mentioned that after similar operations many leaves formed leaflets which probably really were those normal leaflets which had not arisen previously. These leaflets were formed from the edges of the leaf in the normal manner, and together with those formed previously they did not add up to more than seven.

True regenerations after similar horizontal cuts were obtained in four other leaves in some earlier experiments. Two of these leaves were deprived of all their leaflets (presumably five) at the P_5 stage, and one of them subsequently formed seven more leaflets, while the other formed eight more. Another was deprived of its five leaflets at the P_4 stage and formed six more; and still another was deprived of its three leaflets at the P_3 stage and formed eight more. The numbers of leaflets show that some were regenerated, but unfortunately in these early experiments no more details were recorded.

Another leaf that regenerated well (no. 6, Fig. 4*a–e*) was one in which at the P_6 stage all seven leaflets were cut or snapped off separately at their insertions, and

subsequently five more good leaflets were formed, and a sixth which did not survive. It can be seen that five of the regenerated leaflets are inserted terminally and almost at the same level, so that they must have been regenerated from the apical end of the leaf, and not from its edges where most of the original leaflets were inserted.

Fig. 3. Successive transverse sections from above downwards of a P_5 leaf which regenerated after a horizontal cut. Wound scars are shown in black, and in *b* moribund tissue is shaded with lines. *s*=stipule. ×51.

The same is true of another P_6 leaf (no. 7, Fig. 5*a–c*) in which the seven leaflets were removed together by sloping cuts, such as are shown in Fig. 1*b*. This leaf regenerated two good leaflets marked 1 and 2 from its apical end, and an incomplete one marked 3.

A last P_6 leaf, no. 8, from which the seven leaflets were removed separately, also regenerated two leaflets from its apical end and apparently a third one imperfectly formed.

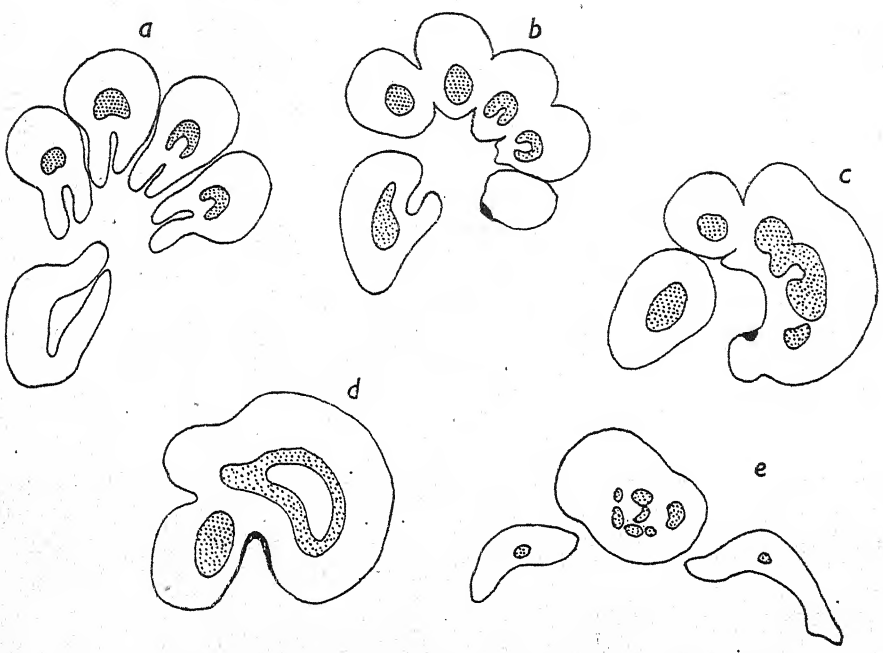

Fig. 4. A P_6 leaf which regenerated after the leaflets were removed separately. ×35.

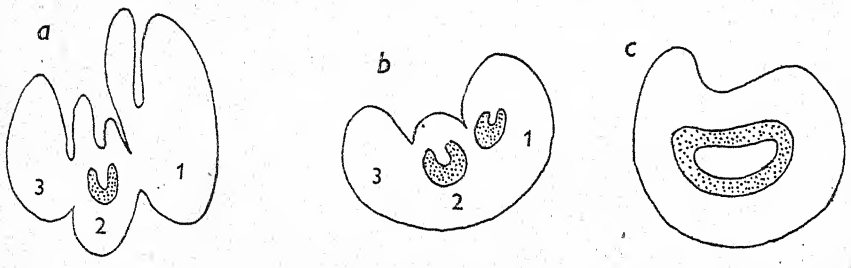

Fig. 5. A P_6 leaf which regenerated after the leaflets were removed by sloping cuts. ×27.

It may be added that two at least of the leaves that were split in a radial plane must have regenerated before forming leaflets. For in these leaves, which were both split at the P_2 stage, each half developed a group of five leaflets, which were orientated nearly in a ring—much more nearly so than those of a normal leaf. One of these leaves is illustrated in Fig. 6*a–d*, which should be compared with Fig. 2, and the other was similar.

It is probable that the leaves of many other species would be found to regenerate if they were to be operated upon at the youngest stages. Indeed leaves that form leaflets in acroscopic sequence would really be more promising than those of the lupin.

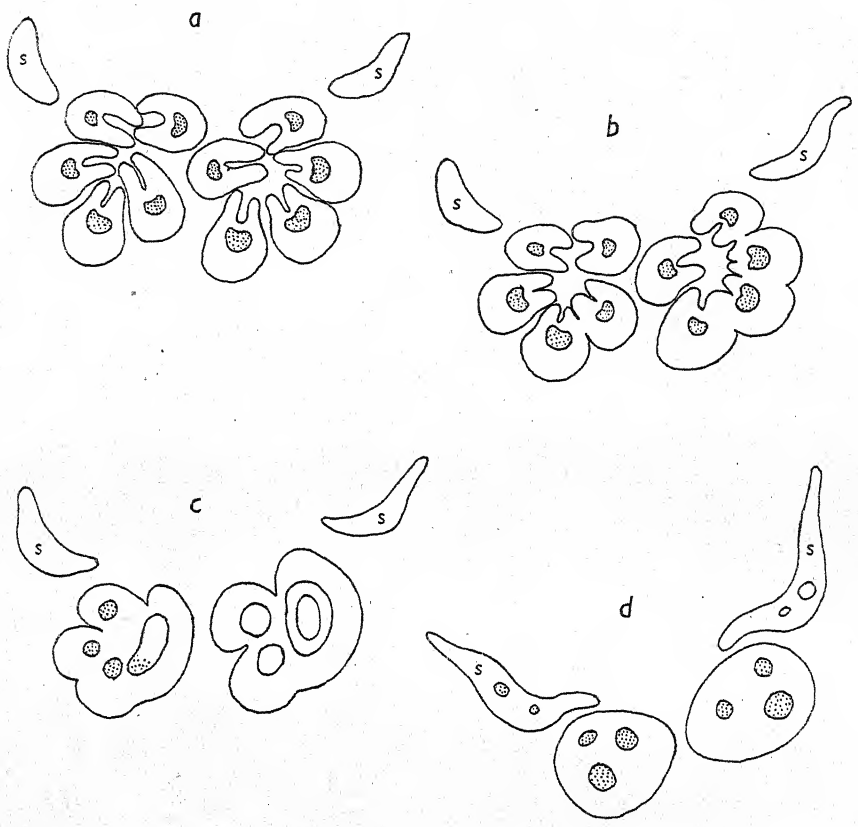

Fig. 6. A P_2 leaf which regenerated after being split in a radial plane. *s*=stipule. ×41·5.

SUMMARY

The very young leaf-primordia of seedlings of *Lupinus albus*, up to the age of the sixth visible primordium at least, can regenerate new leaflets when the original ones are removed. The new leaflets often arise in positions different from those of the old ones. The leaf-primordia can also regenerate when split longitudinally.

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STUDIES IN *VIOLA*

III. AN ACCOUNT OF THE INHERITANCE OF CERTAIN CHARACTERS IN THE PROGENY OF PLANTS OF A WILD POPULATION OF *VIOLA* HYBRIDS

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1. Introduction

This is the third paper of a series of studies in *Viola*, the chief object of which is to investigate the cytology of a wild population of hybrids between *V. tricolor* L. and *V. lutea* Huds. in order to attempt to discover the survival value of various cytological types, and the action of selection on them. By considering the morphology and the inheritance of various characters, coupled with the meiotic cytology and chromosome numbers, it was hoped to be able to indicate the status of the population in cytogenetic terms.

In 1936 the writer began a detailed cytogenetic study of another wild hybrid population of *Violas* which it is hoped to carry to three or four generations. Thus the present research has served as an introduction to this more controlled and extensive study, and has indicated the broad outlines of investigation. Certain general conclusions have been drawn from the data presented in the three papers.

For purposes of revision I may state that the original wild population grew on the banks of the river Tyne near Bywell, Northumberland. Seeds were taken from a plant of this population and named V.B. 15. At the same time seeds were taken from two other plants, V.B. 18 and V.B. 19. These two plants had originally formed part of the wild population but had been transplanted to a nearby garden separated from the population by a steep grassy slope and a field. The seeds were sown immediately, but only those of V.B. 15 and V.B. 18 germinated. The plants thus obtained were named V.B. 15.1, V.B. 15.2, etc., and V.B. 18.1, V.B. 18.2, etc.

Later a collection of small seedlings was obtained from the garden mentioned above. These seedlings grew around and about the plants V.B. 18 and V.B. 19. The garden is sufficiently isolated from the wild population to warrant the assumption that they were the products of open pollination between V.B. 18 and V.B. 19. They were numbered V.B. 25–67.

In the first paper of this series (Fothergill, 1938) a detailed cytological investigation of twenty-two random samples of the wild population was made. In meiosis, univalents, bivalents, trivalents, and quadrivalents were found, but the bivalents were in the great majority. Orientation of the chromosomes on the metaphase spindle was often irregular, and many of the univalents lagged behind the others.

Some of these split and some were eliminated. The result was that the gametic chromosome number varied from 17 to 27, chiefly 23 to 26. One plant showed $n=13$. The tendency of the wild population seems to be to produce a majority of plants with chromosome numbers of from $2n=48$ to $2n=52$, resembling in some respects Kihara's (1919, 1921) and Watkin's (1930) wheat hybrids, for $2n=48$ is the chromosome number of *V. lutea* while that of *V. tricolor* is $2n=26$. The increase is due to the splitting of univalents (vide also Clausen, 1931), some of which become incorporated in the daughter nuclei.

A wild population such as this is in a state of flux, new and untried combinations of chromosomes are being submitted to the test of natural selection leading to the establishment of a secondary balance. Where this is not yet attained, sterility results, and this is shown by the presence of diads, triads, and pentads instead of the normal tetrads, and also by the occurrence of aborted flowers.

The second paper (Fothergill, 1939) deals with these aborted flowers. They are usually shrunken and lack much tissue; the vascular supply is greatly reduced and the sporogenous tissue is structureless. Two such plants, however, indicated that meiosis was extremely irregular while another showed an abnormally high chromosome number of $2n=58$.

It was suggested that lack of chromosomal balance, due to hybridity, had upset the balance of factors responsible for metabolism, and thus supporting the theory of R. O. Whyte (1930) that a reduced level of nutrition in the flower produces abnormalities. In these cases competitive development of floral parts does not enter into it.

Extra-nuclear bodies were described, and while these are essentially similar to the extra-nuclear nucleoli of other writers, it was shown that they have no connexion with the nucleolus, and are possibly excretory products of a tannin-like nature. Finally, an unusual abnormality in the form of extremely shortened somatic chromosomes was found to be quite common in hybrids with a very irregular state of meiosis, or with one or other of the reproductive parts aborted.

In this paper the writer proposes to examine the morphological characteristics and their inheritance in the progeny of some plants of the wild hybrid population.

2. DESCRIPTION OF ASSUMED PARENT PLANTS

The criterion of hybridity in modern cytogenetics is heterozygosity. The more heterozygous an organism the greater the variation in the quality of its gametes and in the morphological characters of its descendants. Genotypical heterozygosity is shown by a study of meiosis in the plants concerned, while phenotypical segregations of distinct characters show directly the results of previous crossings. The irregular cytology of this wild population, and the variations of the progeny of some of them furnishes evidence of the heterozygosity of their parents. The appearance of the plants gives all the confirmatory evidence needed to show that they are hybrids between *V. tricolor* L. and *V. lutea* var. *amoena* Henslow. This variety differs from *V. lutea* Huds. only in the possession of blue-violet flowers.

In the following descriptions the letter (T) after a character indicates that it is typical of *V. tricolor*, while (L) indicates that the character is typical of *V. lutea*.

V.B. 19 (*original*)

Plant fairly tall, slightly branched (T). Leaves very large, obtuse, crenate, glabrous (L), ovate-lanceolate. Stipules digitate (L), large, glabrous (L), middle lobe rather spathulate (L), entire (L). Peduncles very long (L) and slender ascending at an acute angle to the stem (T). Flower large (L), longer than broad (L), spur slightly longer than the sepaline appendages (T); sepals broad, linear and with a scarious margin (T); appendages large and long (T), dentate (T); uppermost petals diverge (L), blue, lateral and bottom lighter blue (T). The ratio of contrasting characters is 9L : 7T, and the plant resembles *V. lutea* more than *V. tricolor*.

V.B. 18 (*original*)

Plant very large, unbranched (L). Leaves very large, ovate to ovate-lanceolate, crenate, hairy (T). Stipules very large, digitate (L), middle lobe slightly spathulate, entire (L). Peduncles very long, slender, ascending at an acute angle to the stem (T). Flower very large (L), much longer than broad (L); spur longer than the appendages (L); sepals conical (T), with a scarious margin (T); appendages large, dentate (T); uppermost petals diverge (L), all petals blue (L). The ratio of contrasting characters is 9L : 5T and the plant is intermediate between *V. lutea* and *V. tricolor*.

V.B. 15 (*original*)

Plant medium-sized, unbranched (L). Lower leaves ovate, intermediate ovate-lanceolate, upper lanceolate-ovate, all crenate, hairy (T). Stipules fairly large, digitate (L), spathulate (L), entire (L), all lobes hairy (T). Peduncles very long and slender (L). Flower very large (L), much longer than broad (L); spur longer than the appendages (L); sepals conical, glabrous (L), with a scarious margin (T); appendages broad and dentate (T); uppermost petals diverge (L), purple, lateral and bottom petals deep blue but the lowermost one is rather paler.

The ratio of contrasting characters is 10L : 5T, and the plant resembles *V. lutea* more than *V. tricolor*.

3. Description of the progeny and their chromosome numbers

Nine pairs of characters of the progeny of the plants V.B. 15, V.B. 18, and the plants V.B. 25–67 are given below. These characters have been chosen because of the fairly easily discernible differences between them. They are taken from representative parts of the plants such as leaves, stipules and flowers. In order to shorten descriptions of this type numbers have been used throughout for each character: the following are necessary:

Leaves.

1 glabrous (L).

2 hairy or slightly hairy (T).

Stipule.

3 digitate to digitate-pinnate (L).
4 pinnate to pinnate-digitate (T).
5 spathulate middle lobe (L).
6 non-spathulate middle lobe, linear (T).
7 entire middle lobe (L).
8 crenate or slightly crenate middle lobe (T).

Flower.

9 sepals with a scarious margin (T).
10 sepals with a non-scarious margin (L).
11 uppermost petals diverge (L).
12 uppermost petals overlap (T).
13 spur shorter than, or equal to, the sepaline appendages (T).
14 spur longer than the sepaline appendages (L).
15 sepaline appendages small, slender (L).
16 sepaline appendages large, broad (T).
17 sepaline appendages dentate (T).
18 sepaline appendages entire (L).

As before the letters L and T are used to indicate whether a character resembles *lutea* or *tricolor*. The ratio of these is given after the formulae, and it indicates quite clearly the general trend of the hybrid, whether it is intermediate between *V. tricolor* and *V. lutea*, or resembles one more than the other. The total frequencies of the characters as distributed among all the plants are seen in Table 1. Thus if we say that a plant possessing twice, or more than twice, as many characters of one of the original parents than of the other, resembles that plant, and all other combination (i.e. of L and T) are intermediate, then 50·72 % of the plants are intermediate between *V. lutea* and *V. tricolor*, 43·43 % are *lutea*-like, while only 5·79 % are *tricolor*-like.

Descriptions

V.B. 15.	1 2, 3, 5, 7, 9, 11, 14, 16, 17,	1·25 : 1	52
	10 2, 4, — 7, 9, 11, 13, 15, 17,	0·6 : 1	52
	11 1, 3, 6, 7, 9, 11, 13, 16, 17,	0·8 : 1	52
	14 2, 3, 5, 7, 9, 11, 13, 16, 17,	0·8 : 1	46
	25 2, 4, 5, 7, 9, 12, 14, 16, 17,	0·5 : 1	50
	26 2, 3, — 7, 9, 11, 14, 15, 18,	3·0 : 1	58
	27 2, 3, 5, 7, 10, 11, 14, 15, 18,	0·8 : 1	51
	28 2, 3, 5, 7, 9, 11, 13, 16, 17,	0·8 : 1	48
	30 2, 3, 6, — 9, 11, 14, 16, —	1·0 : 1	48
	31 2, 3, — 7, 9, 11, 14, 16, 17,	1·0 : 1	50
	33 1, 4, 5, 7, 9, 12, 14, 15, 17,	1·25 : 1	48
	34 2, 3, 5, 7, 9, 12, 13, 16, 17,	0·5 : 1	52
	36 2, 3, 5, 7, 9, 11, 14, 16, 17,	1·25 : 1	48
	38 2, 4, 5, 7, 9, 11, 14, 16, 17,	0·8 : 1	48
	43 2, 3, 5, 7, 9, 11, 13, 16, 17,	0·8 : 1	50
	44 1, 3, 6, 7, 9, 12, 13, 15, 18,	1·25 : 1	48
	45 1, 3, 5, 7, 9, 11, 14, 16, 17,	2·0 : 1	48
	46 1, 3, 6, 7, 10, 11, 13, 15, 18,	2·0 : 1	48
	47 1, 3, 5, 7, 9, 11, 13, 16, —	2·5 : 1	48
	48 2, 4, 5, 8, 10, 11, 13, 15, 17,	0·8 : 1	45
	51 2, 3, 5, 7, 9, 11, 14, 15, 17,	2·0 : 1	—
	53 1, 3, 6, 7, 10, 11, 13, 15, 17,	1·66 : 1	52
	54 2, 3, 5, 7, 9, 11, 13, 16, 17,	0·8 : 1	52

V.B. 18.	2	2,	3,	5,	7,	9,	11,	14,	16,	17,	2·0 : 1	52
	3	1,	3,	6,	7,	9,	11,	13,	16,	17,	0·8 : 1	45
	4	2,	3,	5,	7,	9,	11,	14,	16,	17,	1·25 : 1	42
	5	1,	3,	5,	7,	9,	12,	13,	16,	17,	0·82 : 1	48
	6	2,	3,	5,	7,	9,	11,	14,	15,	—	3·0 : 1	52
	7	2,	3,	5,	7,	9,	12,	14,	16,	17,	0·8 : 1	52
	8	1,	3,	5,	7,	9,	12,	14,	15,	17,	3·0 : 1	27
	13	2,	3,	5,	7,	10,	11,	13,	16,	17,	1·25 : 1	54
	14	2,	3,	5,	7,	10,	12,	14,	16,	17,	1·25 : 1	54
	18	2,	3,	5,	8,	9,	11,	14,	15,	17,	3·0 : 1	48
V.B. 25–67.												
V.B.	31	2,	3,	5,	—	—	12,	14,	16,	17,	1·0 : 1	48
	32	2,	4,	5,	—	9,	11,	14,	15,	18,	1·66 : 1	49
	33	2,	3,	5,	—	9,	11,	14,	15,	18,	3·5 : 1	43
	34	2,	3,	6,	—	—	11,	14,	15,	17,	1·66 : 1	—
	35	2,	3,	5,	—	9,	11,	14,	16,	18,	2·1 : 1	48
	36	1,	3,	5,	—	—	11,	13,	15,	17,	3·1 : 1	44
	37	2,	3,	5,	—	9,	11,	14,	16,	17,	1·25 : 1	44
	38	2,	3,	5,	—	—	11,	13,	15,	18,	3·1 : 1	44
	39	1,	4,	5,	—	—	11,	14,	16,	17,	1·33 : 1	46
	40	1,	3,	5,	—	9,	11,	14,	15,	18,	8·0 : 1	—
	41	2,	3,	5,	—	9,	11,	14,	15,	17,	2·1 : 1	44
	42	2,	3,	5,	—	9,	11,	14,	16,	17,	1·25 : 1	—
	43	1,	3,	5,	7,	—	11,	14,	15,	17,	7·0 : 1	—
	44	2,	3,	5,	8,	—	11,	14,	16,	17,	1·0 : 1	44
	45	1,	3,	5,	7,	9,	—	14,	16,	17,	2·0 : 1	—
	46	2,	4,	6,	7,	9,	11,	14,	16,	17,	0·5 : 1	—
	47	2,	3,	5,	7,	9,	11,	14,	15,	18,	3·5 : 1	46
	48	2,	3,	5,	7,	9,	11,	14,	16,	17,	1·25 : 1	—
	49	2,	3,	5,	7,	9,	11,	—	16,	17,	0·8 : 1	—
	50	1,	3,	5,	7,	9,	11,	14,	15,	17,	3·5 : 1	48
	51	2,	3,	5,	7,	—	11,	13,	15,	17,	1·66 : 1	24
	52	2,	3,	5,	7,	—	11,	13,	15,	—	2·0 : 1	44
	53	2,	3,	5,	7,	9,	12,	13,	16,	17,	0·5 : 1	50
	55	1,	4,	5,	7,	9,	12,	13,	15,	17,	0·75 : 1	44
	56	2,	3,	5,	7,	9,	12,	14,	15,	18,	2·1 : 1	49
	57	2,	3,	5,	7,	9,	11,	14,	15,	17,	2·1 : 1	46
	58	2,	3,	5,	7,	—	11,	14,	16	—	2·5 : 1	46
	59	1,	3,	5,	7,	9,	11,	13,	16,	18,	2·0 : 1	42
	60	2,	3,	5,	7,	9,	11,	14,	16,	17,	1·25 : 1	48
	61	2,	3,	5,	7,	9,	11,	14,	15,	18,	3·5 : 1	47
	62	2,	3,	5,	7,	—	11,	13,	15,	17,	2·5 : 1	42
	63	2,	3,	6,	7,	9,	11,	14,	16,	17,	0·8 : 1	44
	64	2,	3,	5,	7,	9,	11,	14,	15,	—	3·0 : 1	46
	65	1,	3,	5,	7,	—	11,	14,	15,	17,	7·0 : 1	44
	66	2,	3,	6,	7,	9,	12,	14,	15,	17,	0·8 : 1	44
	67	2,	3,	5,	7,	9,	11,	14,	15,	17,	2·0 : 1	48

The somatic chromosome number of each plant is also given at the end of each description. No connexion could be found between the chromosome number of any plant and the possession by it of certain features, or between the ratio of *lutea* and *tricolor* characters. From Table 2 it is seen that the somatic chromosome number varies from 24 to 58. 23·4% of the plants possessed 48 chromosomes: this was the peak number. If we omit such aneuploid numbers as $2n=47$ or 51, etc., then an almost regular curve of decreasing and increasing chromosome numbers with 48 as the peak can be drawn. An almost equal number of plants show $2n=50$ and $2n=46$; $2n=52$ and $2n=44$; $2n=54$ and $2n=42$; and, finally, $2n=58$ and $2n=30$. It was noticed, however, that among the plants V.B. 25–67 the peak number was $2n=44$, i.e. 35·7% possessed this number. Further, it soon became obvious that these plants differed in their inheritance of characters from either V.B. 15 or V.B. 18. It has already been suggested (Fothergill, 1938) that these are

Table 1. *Total frequencies of the distribution of the characters of the progeny V.B. 15, V.B. 18, and of V.B. 25–67*

Ratio of L : T	0·5/1	0·6/1	0·75/1	0·8/1	1/1	1·25/1	1·33/1	1·66/1	2/1	2·5/1	3/1	3·5/1	7/1	8/1	Total
No. of plants	4	1	1	13	4	11	1	4	14	3	5	4	2	2	69
4 = 5·79 % *tricolor*-like		35 = 50·72 % intermediates							30 = 43·43 % *lutea*-like						

Table 2. *Total distribution of somatic chromosome numbers of the progeny of V.B. 15, V.B. 18, and V.B. 25–67 (adapted from Paper I, Table 3)*

Chromosome no.	24	27	30	40	42	43	44	45	46	47	48	49	50	51	52	53	54	58	Total
No. of plants	1	1	1	2	3	1	10	2	8	1	18	3	10	2	9	1	3	1	77
No. of plants %	1·3	1·3	1·3	2·6	3·9	1·3	13·0	2·6	10·4	1·3	23·4	3·9	13·0	2·6	11·7	1·3	3·9	1·3	

the plants which are naturally selected under wild conditions due to cytological instability. There may also be some connexion then between the differences in the inheritance of these plants and the occurrence of a large proportion of low chromosome-numbered plants among them.

4. Segregation of characters

It is apparent from these descriptions that all the plants raised possess characters of both *V. lutea* and *V. tricolor*, and that any combination of them can exist (in the colour of the petals as shown below, for example). Unlike Clausen's experience (1926) of crossings between *V. tricolor* and *V. arvensis* neither of the original species has been recovered in its entirety. The plants as a whole tend to resemble *lutea* more than *tricolor*, or are intermediate between them. In this respect they agree with the plants of the natural wild population in which a random sampling showed 54·5% to be more or less intermediate, 45·5% more *lutea*-like, while only two plants were found which showed more *tricolor* than *lutea* characters, but even these were best described as intermediate types (Fothergill, 1938, Table 1).

The garden pansies first arose from a cross of *V. lutea* and *V. tricolor* and they resemble *lutea* to a much greater extent than *tricolor* (Wittrock, 1897). Thus the appearance of these plants agrees with expectations.

The difficulty of drawing conclusions from the progeny studies of wild populations lies in the fact that only one of the immediate parents is known. No doubt this accounts for the small amount of work which has been done on the cytogenetics of such populations. The author feels that, in the present state of knowledge, it is justifiable to apply established cytogenetic principles to a study of wild uncontrolled populations, and to draw conclusions directly from such wild material. For, after all, theoretical observations based on a study of controlled material do not necessarily reflect the actual state of affairs in nature. The behaviour of the plants V.B. 25–67 illustrates this point.

In the field pollination is haphazard and unknown. The seeds from any chosen plant may have been obtained from flowers that were selfed or crossed by natural agents, or both of these may have occurred. The plants of this wild population certainly indicate that they are hybrids, both by their meiotic cytology and by their morphological appearance. Hence, as the population is very heterogeneous, whether selfed or crossed, the chances are that the parents are heterozygous. Segregation then should normally occur, even in the unlikely event of the pollen being all alike.

Bearing these limitations in mind, a simple analysis of the contrasted characters of the progenies was made. A certain amount of information was thus obtained but, due to the rather small number of plants involved, a complete genetic analysis was not possible. The presence of certain genes controlling specific characters has at least been indicated, and the information obtained will serve as the basis of further studies. There are indications that some characters are inherited in a simple Mendelian fashion, in other cases the inheritance is more complex.

Table 3 gives a summary of the inheritance of the chosen characters by presenting the probabilities in terms of a 3 : 1 ratio. If we take the parents V.B. 18,

V.B. 15, and V.B. 25–67 separately, then the smaller the number of plants examined the more truly does the ratio approximate to a true 3 : 1. In column 3 all the ratios are 3 : 1, in column 5 all but one are 3 : 1, while in column 7 only three cases of this ratio are found. When grouped together this ratio is obtained in more than half the number of cases. As was demonstrated for the actual wild population cytological irregularities in gametogenesis are common, and hence similar disturbances in these plants may possibly have interfered with the production of normal Mendelian ratios. It has already been suggested that there may be some connexion between this and the very large proportion of low chromosome-numbered plants among V.B. 25–67, i.e. among those in which the 3 : 1 ratio is most interfered with.

Table 3. *Summary of the inheritance of characters in terms of the probabilities*

Character	No. of plants V.B. 18	*P*	No. of plants V.B. 15	*P*	No. of plants V.B. 25–67	*P*	Total no. of plants	*P*
2 × 1	10	0·72	23	0·55	36	0·91	69	0·91
3 × 4	10	—	23	0·66	36	**< 0·05**	69	**< 0·05**
5 × 6	10	0·27	20	0·91	36	**< 0·05**	66	0·07
9 × 10	10	0·72	23	0·31	24	**< 0·05**	57	**< 0·05**
14 × 13	10	0·72	23	**< 0·05**	36	0·91	69	0·07
11 × 12	10	0·27	23	0·31	36	**< 0·05**	69	0·23
16 × 15	10	—	23	0·13	36	**< 0·05**	69	**< 0·05**
17 × 18	9	—	21	0·53	33	0·79	63	0·43

Note. *P* represents the probability of the ratio being in agreement with a Mendelian 3 : 1. Those in heavy type represent odds of more than 19 : 1 against this hypothesis. The nearer *P* approaches unity the greater the chances of agreement with the hypothesis and hence of the operation of a single gene. Intermediate values probably indicate the operation of other, or polymeric factors.

The chief cytological irregularity is the lagging of split and unsplit univalents in meiosis. It is the splitting of these bodies which has forced up the chromosome number of the hybrids. Thus many of the chromosomes will be present in duplicate or triplicate. Regular allopolyploids, as many of these plants seem to be, should give a more or less normal diploid ratio, but the chance elimination of univalents would upset this. The low chromosome-numbered plants are precisely those in which elimination is at a maximum.

Owing to the fairly small number of plants dealt with it is not advisable to look for further evidence of the operation of polymeric genes. Clausen (1931) demonstrated their presence for flower colours in similar hybrids of *Viola*.

A more detailed account of the inheritance of these characters reduced to the simplest terms is given below:

A. *The leaves*

Hairy (2) *and glabrous* (1) *leaves.* **Hh × Hh.**

V.B. 18 and V.B. 15 both have hairy leaves unlike those of V.B. 19 which are glabrous. The probability that this character is controlled by a single gene (**H**) is high, hairy being dominant to glabrous. This supports Clausen (1931) who finds

that the hispid type of hairiness of *V. rothomagensis* is probably dominant to glabrous.

B. *The stipule*

(*a*) *Digitate* (3) *and pinnate* (4) *forms of stipule.* **Tt** × **Tt.**

V.B. 18, V.B. 15, and V.B. 19 all have digitate stipules. Clausen (1931) in the cross *V. elegantula* × *V. cornuta* finds numerous gradations in the shape of the stipule but segregation occurred. There are gradations in the crosses concerned here, but a random selection shows that the digitate type is clearly marked off from the pinnate type; within each of these types there are minor variations. Digitate is obviously dominant, because of the variation of the character it is likely that there are several genes operating. There are only slight indications that a single gene is responsible.

(*b*) *Spathulate middle lobe* (5) *and non-spathulate middle lobe* (6). **Pp** × **Pp.**

V.B. 18, V.B. 15, and V.B. 19 have a spathulate middle lobe of the stipule. Spathulate lobe is typical of *V. lutea.* Table 3 shows that a 3 : 1 ratio can be expected, while spathulate is dominant. A single gene, **P**, may then be operative.

(*c*) *Entire middle lobe* (7) *and crenate middle lobe* (8).

Segregation for this character was very slight, only two plants (V.B. 15.48, and V.B. 44) were found with a crenate middle lobe of the stipule.

C. *The flower*

(*a*) *Scarious* (9) *and non-scarious* (10) *margin of the sepals.* **Cc** × **Cc.**

The scarious margin of the sepals consists of a thin line of tissue lacking chlorophyll which runs down the edge of the sepals. It is absent in *lutea* but present in *tricolor.* It was present in V.B. 18, V.B. 15, and V.B. 19. Scarious is obviously dominant. With a small number of plants a 3 : 1 is obtained, but as the number of plants increases the probability decreases. Thus a gene (**C**) is present but it is probably only one of several responsible for the inheritance of the character.

(*b*) *Long* (14) *and short* (13) *spur of the lowermost petal.* **Yy** × **Yy**, *and* **Yy** × **yy.**

The petaloid spur of *V. lutea* is very long while that of *tricolor* is scarcely longer than the sepaline appendages from which it protrudes. V.B. 18 and V.B. 15 possessed a long spur, but that of V.B. 19 was only slightly longer than the appendages. There are indications that a single gene (**Y**), dominant for long spur, is present; but as one supposed parent, V.B. 19, possessed a short spur, then this cross would be equivalent to a backcross on the recessive, and the ratio should be 1 : 1. Thus this group is again exceptional.

(*c*) *Diverging* (11) *and overlapping* (12) *uppermost petals.* **Gg** × **Gg.**

Diverging uppermost petals is characteristic of *lutea*, those of *tricolor* overlap. The parents had diverging petals. This character is dominant, but the probability of occurrence of a 3 : 1 ratio is only about four to one. Thus while a single gene (**G**) may be present the inheritance of the character is rather more complex.

(*d*) *Large* (16) *and small* (15) *sepaline appendages.*

Segregation for this character was noticed, and V.B. 15 gave a 3 : 1 ratio, but the other cases showed that the probability that a single gene is operating is small. Neither character is strictly dominant.

(*e*) *Dentate* (17) *and entire end* (18) *of the sepaline appendage.* **Nn** × **Nn**.

All the progeny of V.B. 18 possessed dentate sepaline appendages, but in the other cases a 3 : 1 ratio was obtained. The parent plants possessed dentate appendages. It is probable that a gene (**N**), dominant for the dentate character, is present.

(*f*) *Direction of lateral petals.*

All the Melanium *Violas* possess lateral petals which are directed upwards, while those of the Nominium *Violas* are directed downwards. These two characters serve to distinguish these two sections of the genus. There are two exception in the Melanium series and these are *V. cornuta* and *V. Orthoceras*. It is of interest, therefore, to note that the plant V.B. 41 showed its two side petals pointing downwards and underlapping the lowest petal. The opposite extreme was met with in the plants V.B. 15.10, and V.B. 15.27, where the lateral petals almost completely overlapped and hid the two uppermost petals.

Clausen suggests that the character is controlled by the interaction of several genes. The three aberrants produced above may suggest that possibly the Melanium *Violas* possess several inhibiting genes controlling the upward direction of the petals. Occasional removal of several of these genes, due to cytological irregularity, would tend to over-emphasize the direction of the petals in an upward direction, i.e. the lateral petals would cover the uppermost ones.

(*g*) *Flower colour.*

The inheritance of flower colour in *Viola* is complex. There are no simple self-colours, but a wide range varying from violet, purple, blue, and yellow to white; a mixture of these may be present on the different petals. The ratio of coloured and white flowers was 3 : 1 with a probability of 0·39. This seems to show that the colours are of the same order. The various shades are produced by modifications of a previous, perhaps basic, colour, not by its complete suppression or absence, except in the case of an all white flower. Clausen (1926, 1931) has worked out very extensively the system of genes responsible for the variations of colour in several *Violas*.

He finds the following: **A**, a gene determining the intensity of the violet and dark colours; it forms a polymeric series $\mathbf{A}_1$, ... $\mathbf{A}_6$, all are dominant. $\mathbf{M}_1$, ..., $\mathbf{M}_5$ a series of dominant and partially epistatic genes modifying this colour to violet. $\mathbf{R}_1$ and $\mathbf{R}_2$ two polymeric genes changing reddish colours to violet. **LL** produces yellowness in the side and bottom petals. It is epistatic to violet **W**, a gene having a bleaching effect upon the violet and the bright yellow (changing it to yellowish-white). **Pal**, a bleaching gene. He also finds a gene, **V**, present in *V. Orphanidis* and *V. rothomagensis* producing another type of violet flower. The flower colours here are

explicable if we assume that purple colour (distinct from violet) contains this gene also. It may then be present in either *lutea* or *tricolor*, or both. It is epistatic to **A, M, R,** and **L.** Blue seems to indicate the presence of **L** in a heterozygous state. The bleaching gene **Pal** is inherited in a simple Mendelian fashion and probably bleaches all colours to some extent.

It can be understood that the system of gene reactions set up by these various genes will be very complicated. Table 4 shows that almost every combination of colours has been produced. In this account the colours of the various petals are given in the order uppermost, lateral, and bottom petal. In the progeny of V.B. white, yellowish white, pale yellow and yellow, yellow, deep yellow are the prevalent combinations. The pale yellow colour of the upper and lateral petals contrasted with the deep yellow of the bottom one, and indicates the presence of **L** and the absence of the bleaching genes. In V.B. 15 the ordinary combination of *V. tricolor* is perhaps most realized in the flowers with blue, pale blue, and whitish yellow; here these are in the majority. The gene **A** is present as a recessive, and **L** is heterozygous. The majority of the plants of V.B. 18 are purple, blue, blue (or bluish white). Here the action of the genes **V** and **Pal** are apparent.

Table 4. *Inheritance of flower colour in the wild population*

Phenotype	Genotype	No. of plants
Purple, blue, bluish white Purple, blue, blue	A M R V w Ll Pal	19
Purple, bluish-white, yellowish white	A M R V w Ll Pal	3
Purple, yellow, deep yellow	A M R V w LL pal	1
Violet, blue, blue Violet, blue, bluish white	A M R v w Ll Pal	4
Violet, yellow, deep yellow	A M R v w LL pal	1
Blue, white, white All bluish white	a M R v w Ll Pal	2
Blue, bluish white, whitish yellow	a M R v W Ll Pal	10
Blue, white, yellow	a M R v W Ll pal	3
Yellow, yellow, deep yellow	A M R v w LL pal	9
White, yellowish white, pale yellow	a m r v W LL Pal	11
White, yellowish white, deep yellow	a m r v W LL pal	2

Note. The genic notation is after Clausen, and, except in the case of the gene **LL**, only single factors are given. No polymeric genes are included.

The chief action of the gene **LL** is very noticeable. It first acts on the bottom petal, then the lateral one, and sometimes the upper one. When the uppermost petals show its presence they are usually bright yellow thus indicating the absence of the gene **W**. The first effect of at least one **L** factor is seen in an almost alba flower: white, yellowish white, whitish yellow; this is followed by white, yellowish white, pale yellow, and finally by white, yellowish white, deep yellow. Here the gene **Pal** has no effect, and **W** bleaches the yellow of the lateral petals.

Two curious chimeral flowers were found. In one of them the top half of the uppermost petals was purple while the bottom half was yellow, the side ones were yellow, bottom one deep yellow. In this then the effect of the gene **LL** was noticeable, but, as Clausen has observed, it could not completely affect the upper petals.

In the other flower the top half of the uppermost petal was white, the bottom half was purple, the side petals white, and the lowest one yellowish white. Such a flower was possibly produced by a local somatic dislocation of the gene **AA** in the upper petals.

5. CONCLUSION AND SUMMARY

1. In conclusion, just as among the plants of the actual wild population, neither of the original parents has been recovered in its entirety. This is contrary to the experience of both Clausen (1926) and Kristofferson (1923). The plants obtained by chance crossings and selfings from V.B. 15, V.B. 18, and V.B. 19 show that any combinations of characters can exist among them, but they tend to be either intermediate between *Viola lutea* and *V. tricolor*, or to resemble *V. lutea* to a much greater extent. This agrees with expectations.

2. The chromosome number of these plants varies from $2n=24$ to $2n=58$, but the peak number is $2n=48$. This is also the somatic number of *V. lutea*. There is no connexion between the chromosome number of any plant and the possession by it of any combinations of morphological characters.

3. Omitting aneuploid numbers (14·3 % of the plants possessed an odd number), the fall in chromosome number on each side of the peak was quite regular. However, the series V.B. 25–67 showed the presence of a large proportion of low chromosome-numbered plants. In fact, in this group the peak number was $2n=44$. The effect of natural selection was to a large extent eliminated from operating on this group. They are cytologically irregular, and it is suggested that this, coupled with the occurrence of such a large number of low chromosome-numbered plants, has upset the normal expected Mendelian ratios.

4. An analysis of contrasting specific characters showed that segregation usually occurred. The presence of the following specific genes is at least indicated. These often Mendelize, and some of them are possibly present as polymeric factors: **H**, a dominant gene producing hairiness of the leaves; **P**, a dominant gene for spathulate middle lobe of the stipule; **Y**, a gene for long and slender petaloid spur, dominant; **N**, a gene dominant for dentate sepaline appendages.

5. Several, or polymeric, genes probably control the inheritance of the characters: (*a*) digitate (dominant) and pinnate stipules (basic gene **T**); (*b*) scarious (dominant) and non-scarious margin of the sepals (basic gene **C**); (*c*) diverging (dominant) and overlapping uppermost petals (basic gene **G**). Segregation for entire and crenate middle stipule lobe was very slight, and, while segregation of large and small sepaline appendages occurred, the inheritance is not simple.

6. The inheritance of the petal colours is complex; they have been tabulated assuming the presence of the genes described by Clausen. It is suggested that the gene **V** is present in these plants, i.e. in *Viola tricolor* and *V. lutea*.

7. Three curious aberrants affecting the direction of the lateral petals and two chimeral flowers, are described.

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ON ABNORMAL FLOWERS OF *PRIMULA VULGARIS* HUDS. GROWN IN CEYLON

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(With 1 figure in the text)

Under Ceylon conditions, the common primrose (*Primula vulgaris* Huds.) is known to flower normally in cultivation at high elevations (*c.* 6000 ft.) in regions of high rainfall. A case was brought to the writer's notice in which a number of plants produced abnormal flowers after transplanting to a lower elevation. The nature of the abnormalities and the circumstances of their origin appear to merit a short discussion.

Description

The flowers originally examined were characterized by the pronounced phyllody of the calyx. The free teeth of the calyx were enlarged into miniature leaves up to 19 mm. in length and 12 mm. wide, forming a rosette with a maximum spread of 40 mm. The tubular portion beneath appeared unaffected. Not all the sepals in one flower were equally enlarged; usually one or two of the leafy structures were somewhat smaller and at the same time asymmetrical in outline (Fig. 1 A, B). All exhibited the prominent venation and other superficial characters of the normal foliage leaves. The margins tended to be revolute and the apex was obtuse, slightly emarginate, or acuminate and reflexed. On a later occasion a batch of flowers became available in which the phyllody was less distinctive, the leafy calyx teeth not projecting beyond the corolla. Here too the individual sepals appeared more variable, those least affected ending merely in a tapering point, rather shorter in length than the tubular part of the calyx, and devoid of foliar characteristics (Fig. 1 C, D). Few of the sepals were as much modified as any of those in the first batch of flowers collected from the same group of plants about a fortnight previously. However, no flowers were seen in which a tendency to phyllody was not clearly recognizable in at least part of the calyx. About one-quarter of the flowers examined were tetramerous and in these the phyllody was well pronounced.

Certain flowers exhibited additional abnormalities affecting the stamens and corolla. In one instance, the corolla was apparently lacking but was observed as a brown, persistent sheath over the ovary. In another, the petals while still concealed within the calyx tube were normal in colour and texture but the stamens were missing, no trace of these being discernible. In one flower with pentamerous calyx and five normal stamens, four petals only were present, the development of the fifth segment having ceased at the point of insertion of the corresponding stamen, leaving a gap in the corolla tube above this level. Instances of phyllody of the corolla were

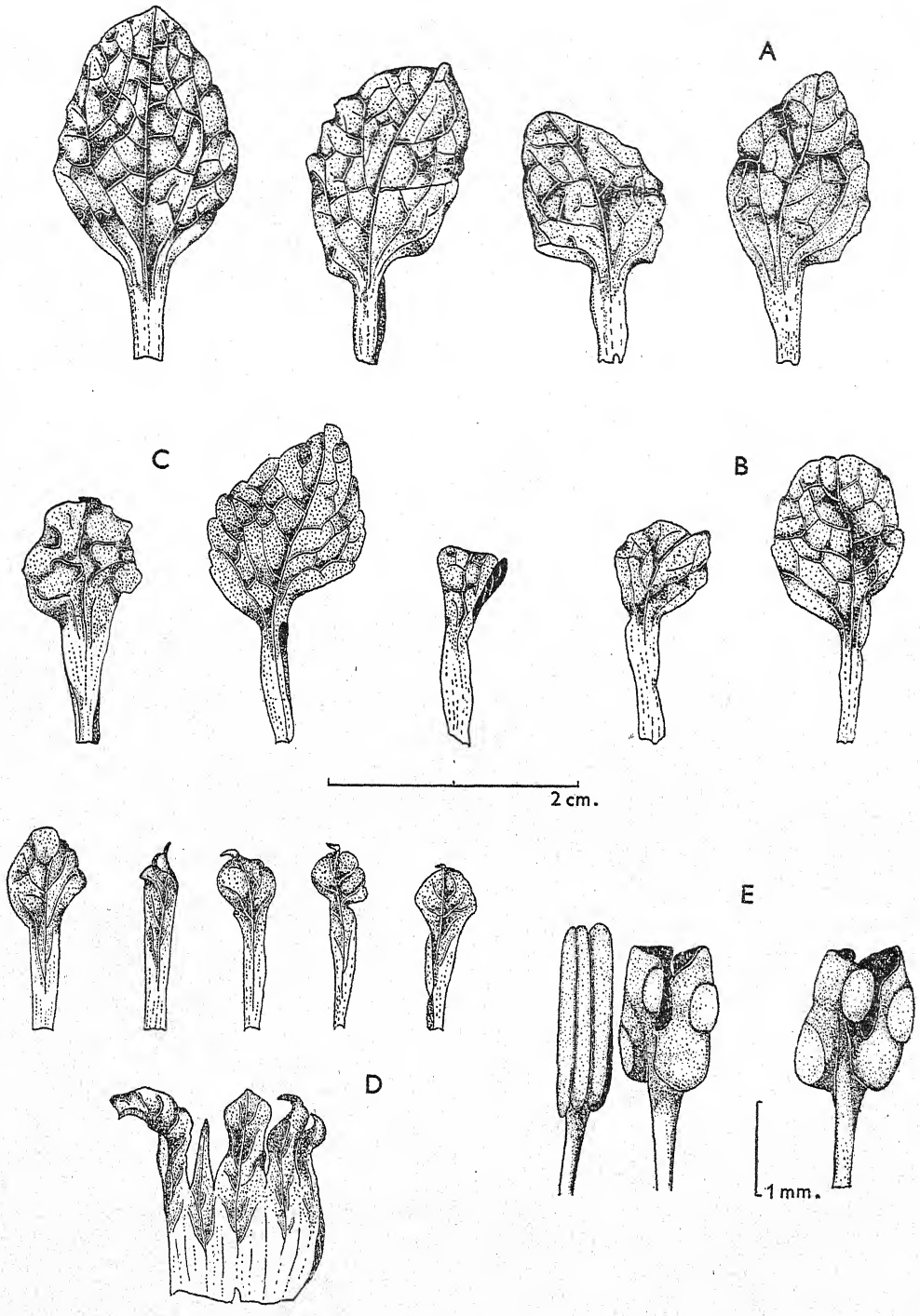

Fig. 1. A–D. Dissections of leafy calyces of *Primula vulgaris*. A. From a tetramerous flower: phyllody well-marked. B. Pentamerous: showing variation between individual sepals. C, D. Two calyces from flowers examined a fortnight later than the above: leafy calyx-lobes not projecting beyond the corolla. E. Two drawings of an abnormal stamen showing bilobed, cup-shaped anther with small, widely separated pollen sacs. On left, seen from within the corolla tube, with adjacent normal stamen.

reported, but no specimens showing this condition were available for examination. Two abnormalities were observed affecting a single stamen without any corresponding modification of the corolla. In one pentamerous flower there were four normal stamens and one in which the anther was elongated and spirally twisted. In the other flower, also pentamerous, only three normal stamens had developed, the fourth being absent and the fifth having an enlarged, cup-like and bilobed anther with widely separated pollen sacs (Fig. 1 E). Apart from these abnormalities, and the complete absence of stamens in one flower, already noted, the essential organs appeared unaffected. Pollen was produced abundantly and the ovaries contained normally developed ovules, in some of which fertilization had apparently occurred. All the flowers examined were of the long-styled 'pin-eyed' type.

DISCUSSION

Examples of the occurrence of phyllody in the calyx are fairly numerous in recent literature. The condition is described most frequently among families where the calyx is polysepalous, and its occurrence in *Primula* seems to indicate that Salisbury's (1931) opinion as to the identity of sepal and leaf base in *Ranunculus parviflorus* holds good also for the components of a gamosepalous calyx. The different sepals are commonly affected in varying degree. Thus, in the case of the rose varieties described by Cook (1926), the tendency to phyllody was much reduced beyond the middle of the third sepal in order of development, the first and second sepals being markedly leaf-like, while the fourth and fifth were but little modified. Schlösser (1936), on the other hand, described a wild race of tomato (*Lycopersicum* sp.) in which leafy sepals were developed only by the single flower of the first-formed inflorescence, the later inflorescences being normal. Among families with a gamosepalous calyx, a successional tendency to phyllody would seem more likely to affect differently the behaviour of complete flowers or inflorescences than to distinguish between the individual members of which the calyx is composed. Such an effect is suggested, in the specimens under consideration, by the marked difference in the degree of phyllody exhibited by the two batches of flowers examined.

In the absence of experimental investigation, for which no opportunity is available, only tentative suggestions as to the cause of the abnormalities here described can be put forward. A number of distinct possibilities exists. Thus, phyllody and other vegetative tendencies affecting the inflorescence region are characteristic of certain virus diseases, as for instance 'false blossom' of cranberries caused by *Vaccinium* virus 1. Numerous other examples can be found in Smith's (1937) text-book. Again, the writer (1940) has recently drawn attention to the abnormal occurrence of leafy bracts in the modified inflorescence of *Elymus arenarius* infected by the smut fungus *Ustilago hypodytes*. Instances of phyllody controlled by genetic mutation have frequently been described: the leafy calyx of *Primula sinensis* (Anderson & de Winton, 1935) is probably the best known of these. No fungal infection was found in the specimens examined and while the possibility of virus infection or gene mutation must still remain, a more likely explanation would appear to be found in the abnormal environment under which the plants

were growing and, in particular, to the change in environment following transplantation. The plants were described as having in all probability been brought out from England some years previously and at the original station, at an altitude of 6000 ft., they had flowered normally. The nearest locality at this altitude for which meteorological records are available is the town of Nuwara Eliya (about three miles away, elevation 6170 ft.) where the mean temperature is 59·5° F. with a mean daily range of 14·1° F. (data from Jameson, 1939). They were then transplanted to a new station at 4650 ft. elevation and, on becoming established, commenced to flower abnormally. Judging from the Tea Research Institute's meteorological observations, at 4525 ft., the mean temperature at the new locality would be about 6° higher than the mean at the original station at 6000 ft. In these Ceylon localities, at a latitude of only 7° north of the equator, *P. vulgaris* would almost certainly be subjected to an unfavourable photoperiod and, unless grown in very heavy shade, would experience considerably more than the optimum intensity of sunlight. The suggestion is offered that the relatively low mean temperature at the original locality was sufficiently close to the optimum to permit the occurrence of normal flowering despite the otherwise unfavourable conditions, whereas this balance was upset on transplanting to a new locality where the mean temperature was very slightly higher. At lower altitudes, with still higher mean temperatures, flowering is known to be inhibited completely.

The relationship between photoperiod and temperature has been stressed by Roberts & Struckmeyer (1938) and other recent workers and a number of instances have been recorded of abnormal environmental conditions, and particularly a sudden change to an unfavourable environment, affecting the transition from vegetative to reproductive activity and inducing various degrees of phyllody in flowers. Thus, Cook (1926), quoted above, observed in rose varieties a tendency to develop leafy sepals under late season conditions, while Darrow (1930) postulated an environmental stimulus necessary for the appearance of leafy calyx in the dewberry (*Rubus* spp.). Philp (1933) noted a possible effect of high temperature in inducing various abnormalities, chiefly of the stamens and pistils, in the flowers of *Prunus avium* growing in the hot interior valleys of California, U.S.A. These abnormalities were progressively less frequent as the foothills were ascended and were absent in the coastal valleys, where the summer temperatures were lower. Again, Cochran (1934) noted the effect of high temperatures in inducing teratological modifications in the flowers and fruits of *Capsicum frutescens*. Finally, Burkill (1935) described an abnormal population of *Scilla nutans* Sm. in which supernumerary bracts were developed in the lower part of the racemes and various modifications occurred in the flowers. This 'invasion of vegetative tendencies' into the inflorescence region was associated with the fact that the plants were growing in the absence of shade, the normal shrub layer having been removed. The same author drew attention to some earlier work with *Lamium* spp., in which abnormal flowers were induced by the shock of transplanting from heavy shade to a sunny place. No useful purpose would be served by multiplying these examples, but from the evidence which they afford, it seems likely that similar abnormal behaviour will be found to result from the

attempt to grow temperate species, such as *Primula vulgaris*, under tropical conditions.

SUMMARY

Phyllody of the calyx and other abnormalities are described in flowers of the common primrose (*Primula vulgaris* Huds.) cultivated in Ceylon at an elevation of 4650 ft. Various possible causes of this condition are discussed in the light of recent literature and the suggestion is made that the abnormality was induced by a change in environment resulting from transfer from a higher elevation, where the mean temperature was slightly lower and where flowering had previously been normal.

My thanks are due to Dr C. H. Gadd, to whom the specimens were sent in the first instance, for suggesting the preparation of this note and for his assistance in reading the manuscript.

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NOTE ON THE CULTURE OF FRESHWATER ALGAE

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THE following simple recipes have been repeatedly used here for producing large quantities of certain material for class work. It should be emphasized that the methods are entirely empirical and found by casual experiment without scientific background. Each recipe seems to produce a peculiar environment in which the organism in question can thrive but which most of its competitors and enemies cannot tolerate.

The algae are grown in half wine casks sunk in the soil in a fairly open place, but other vessels will serve very well. If oak wine casks are used, they need long soaking to rid them of wine residues, but then they last for many years. The casks are filled with tap water (which happens to be hard and has been chlorinated), which is enriched by a generous basic addition of a phosphate and a potash salt—say 100 g. of sodium phosphate and of potassium sulphate; it does not matter if more is given. A certain amount of calcareous earth, and also leaves and insects, get into the water, but do no harm if the bulk of the leaves and any corpses large enough to foul the water are removed occasionally. It may be well to add an inoculum of mixed pond mud, but in my experience great numbers of algae have appeared without this.

(1) *Chlamydomonas*. Nitrogen is supplied as ammonium nitrate, say 50 g. at first and 20 g. at intervals of a month. When the water is getting green it should be made anaerobic by the occasional addition of a lump of sugar: possibly a substitute might serve as well. This prevents the growth of plankton-feeding animals and results in the production of a dense population of various large-celled species of *Chlamydomonas* which is stable so long as the supply of ammonium nitrate and sugar are maintained.

It is interesting to note that certain floating water plants, notably *Riccia fluitans* and *Salvinia* sp., which do not always thrive in ordinary or enriched tap water, grow exceedingly well in this enriched anaerobic water.

(2) *Pandorina*. Nitrogen is added at intervals as lumps of dried egg albumen. The water becomes rather smelly but *Pandorina* flourishes as a dominant organism.

(3) *Pediastrum* and *Scendesmus*. Nitrogen is added as potassium nitrate, and the water is made very strongly alkaline by the addition of a pound or more of washing soda. The water should be stirred occasionally or the heavy *Pediastrum* colonies sink and the water is occupied by smaller forms. No less than eighteen species belonging to twelve genera of the Chlorococcales appeared in a single tub with this treatment, but *Pediastrum* and *Scenedesmus* predominate. Sometimes *Eudorina* becomes abundant but no method of stabilizing this has yet been found.

(4) *Euglena viridis*. Nitrogen is added as ammonium sulphate. A purer growth of *Euglena* results if the water is made and kept distinctly acid (at *p*H 4–6) by sufficient additions of sulphuric acid. (The preference of *E. viridis* for ammonium salts is well known.)

Most of these culture solutions have no known relation to the conditions under which the organism occurs naturally. The possibilities for the discovery of further recipes of this sort seem endless; some, such as a stable culture of *Volvox*, would be very useful.

REVIEW

Mechanisms of Biological Oxidations. By D. E. GREEN. $8\frac{1}{2} \times 5\frac{1}{4}$ in. Pp. 181, with 22 figs. in the text. Cambridge: University Press. 1940. 12*s.* 6*d.*

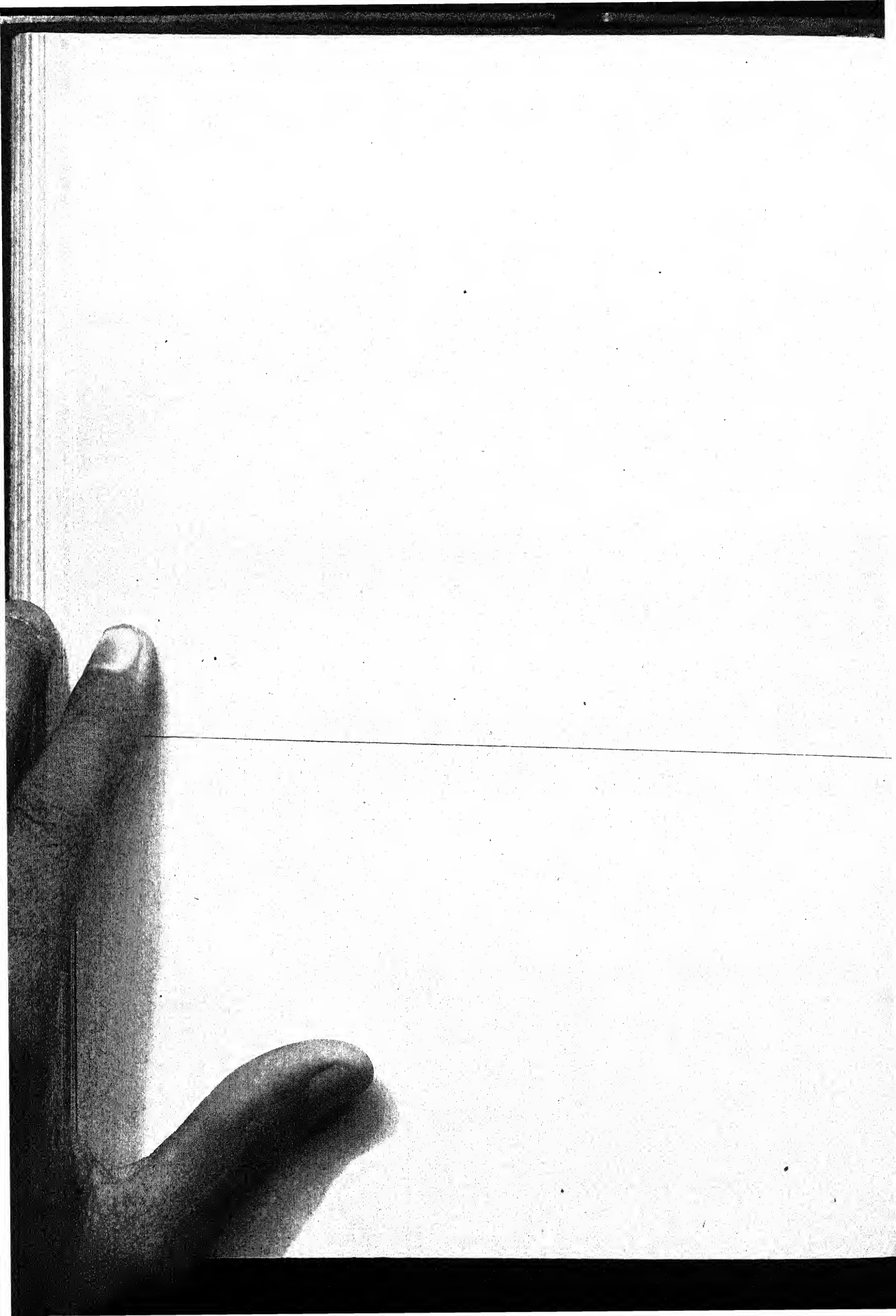
It argues a certain degree of courage to write a book on so rapidly advancing and in some ways controversial a subject as oxidation in living systems. It is a measure of the author's great success that a clear picture emerges of the nature and magnitude of the progress recently made. It would have seemed incredible a decade ago that a purely chemical classification could be successfully applied to a large proportion of the major systems. The chapter headings of this book show how possible it has already become. The brilliant work that has led to the isolation, purification and identification of so many prosthetic groups is here concisely recorded, together with the catalytic powers associated with each, and what is known of the conditions of activity. Questions of kinetics and energy transfer are, of necessity, sparingly treated.

The general treatment is such as to be useful to advanced students and indeed to any non-specialist desiring a knowledge of this intensely interesting branch of biology. The entire range of living tissues is covered; but it is with good reason that the author remarks that the greater part of present knowledge derives from a few animal tissues, special yeasts and bacteria. The omission of the higher plants is significant, and the careful balance preserved in the text emphasizes it. Botanists requiring a little weighting on the plant side might find the summary recently published in this journal (vol. 39, p. 241) a useful supplement.

There are a few unfortunate misprints and slips scattered about the book, especially in equations and formulae, some of them of more than mere typographical importance; which may, however, serve a useful purpose as pinpricks to mental alertness. A more serious shortcoming lies in the indexing which is so inadequate as seriously to impair the book's usefulness as a book of reference. There is, for example, on p. 48 an important definition of the meaning which the author attaches to the term "dehydrogenase" in frequent use throughout the book. The index does not recognize the existence of "dehydrogenase" at all, and such omissions are very plentiful. The whole question of nomenclature is dismissed by the author as one of convention, and this is perhaps the only important case in which he sends the reader empty away.

This book is likely to be of the greatest service to students and to remain so until further advances make it out of date.

W. O. JAMES



OBITUARY

SIR ALBERT CHARLES SEWARD, F.R.S.
1863–1941

THE recent death of Prof. Seward has deprived us of one of the world's great botanists; great not merely in his direct contribution to knowledge, but in character and in the part he played in the progress of biological science generally.

Born in Lancaster, he was educated at Lancaster Grammar School, and St John's College, Cambridge, entering on his University career at the wish of his parents who intended him for the Church. He soon, however, changed to Science, having already as a boy shown the keenest interest in both botany and zoology. He used to say that he was first inspired to devote himself to the study of fossil plants by W. C. Williamson who was giving University extension lectures at the time.

His ability was early recognized, and he was appointed a lecturer in botany. Later he became a Tutor of Emmanuel, and in 1915 he became Master of Downing College: he had already in 1906 been appointed Professor of Botany in succession to Marshall Ward. His period of Vice-Chancellorship in 1924–6 was the climax of this career of double service in Cambridge—to Colleges and to the University.

His scientific publications began with a research note in 1888 and will end with a book on Geology which he had only just finished. During this period of more than half a century he wrote very many important works leading to much general progress, yet the centres of interest in his work were maintained with little change. His interests owed their origin to his own nature and were not imposed on him by the material of his work.

His first considerable work was his Sedgwick Essay on *Fossil Plants as Tests of Climate*, where will be found a particularly happy blend of learning, caution and encouraging speculation. This was followed by his British Museum catalogues of *The Wealden Flora* and *The Jurassic Flora*, each a two-volume work, and during the same period came the first volume of his text-book.

The remaining volumes of his text-book appeared at intervals during the next twenty years: this great work is justly valued for its store of information for research workers, but it may also be recalled that it contains many excellent chapters for students on recent plants considered in relation to fossils, which are written in an easy and spacious style. During this period also, he wrote an impressive series of descriptive papers on floras from all parts of the world, but mostly of Jurassic and Cretaceous age; later, however, he devoted himself more to the northern regions, particularly Greenland where he spent a very happy summer collecting. It is interesting to note that during his last years he had been co-operating with

W. N. Edwards in describing a flora of the Hebrides—this would have been his first long paper on a purely Tertiary flora where dicotyledons predominate.

During his later years he devoted more of his attention to general works, and a little less to description of fossils; yet it is most difficult to distinguish periods in his scientific career, for at all times his interests were so wide as to include the whole of palaeobotany and much of recent botany too. It is true that it was his masterly contributions to our knowledge of the Mesozoic plant world which won him his scientific reputation and seem to overshadow his other work; yet his writings on Palaeozoic, Tertiary and recent plants make an impressive series even when considered alone.

Throughout his writings his preference for certain sides are clear; he liked things that were open and large, and his efforts were directed to making them more open and larger. He chose for the major part of his descriptive work the external form of fossil plants rather than anatomy, and the form visible to the naked eye or hand lens rather than the minute details of miscroscopic study. It is true he wrote some valuable papers on the anatomy of fossil ferns and cycads, but in them the reader may search in vain for the details of vascular bundle behaviour which are the delight and perhaps also the pitfall of the anatomist. He knew that by leaving fine detail aside he might sometimes miss things of interest, but faced as he was with abundant material and limited time he knew he must omit certain sides. Indeed, it is remarkable that he should have been able to devote his mind so largely to research, faced as he was by endless daily problems as Head of a large Botany School and Master of a College. His great energy enabled him to make full use for research of odd half hours between engagements, but few could maintain a piece of research lasting perhaps two years under these conditions.

The fruit of his work was rather to widen the limits of his science by a multitude of minor discoveries than to make spectacular advances in particular directions. His preference for the large led him to maintain in systematic work that when in doubt it was better to unite genera or species than to separate them, and it would indeed be hard to find a more wholehearted 'lumper'. Not only did he unite what others had separated but he showed that he had little patience with the subtle intricacies of rules of nomenclature. His preference for what seemed to him the natural and sensible way led him now and again into controversy but never into ill humour. He was, in fact, always ready to be convinced that what he had previously said was mistaken. He was large-minded in personal outlook; when he criticized a man's work you realized he liked and respected the man; he did not speak of people he disliked.

On the geological side too he followed the broader issues; he was not concerned with using fossil plants to split formations of date horizons, so much as the synthesis of his evidence to point to widespread migrations of species and to climatic change. He was particularly delighted when he could show that a plant that is now rare and confined was once widespread and dominant. The theme of his first piece of general palaeobotanical work, on the use of fossil plants as indicators of past climates, is met in the conclusions of many of his papers.

Noteworthy too is his part in furthering the progress of his science by helping young men to become active workers on fossil plants. Considering his eminence, Seward wrote remarkably few joint papers, but it is significant that a high proportion of those with whom he worked established themselves as palaeobotanists—one might mention Thomas, Sahni and Walton. He had strong views about the part which should be played by the senior author in a joint paper, and he refused to let his name appear unless he had in fact done about half of the groundwork on the material. Far larger indeed is the number for whom he had provided a research problem, and helped continually throughout the progress of their work and in the drafting of their papers; and many came for shorter periods from all over the world for this advice about their work.

For this purpose he was an almost ideal man, as I can testify from personal experience. The younger worker had all the advantages of Seward's vast knowledge and enthusiasm and felt happy in the assurance of his continued loyal friendship and help; yet somehow Seward never dominated the views of others; indeed, he would have thought little of anyone who simply reflected himself and took up no new position in research. He avoided dominating, not so much because of the modesty with which he expressed opinions, as because his self-respect made him have the deepest respect for others. His influence was no doubt fundamental, but it was by his example and not consciously exerted.

Seward was a splendid teacher and a most successful head of the largest Botany School in the country. He thoroughly enjoyed lecturing at all levels, but I believe he liked the elementary lectures best, and certainly it was a joy to hear them. After lecturing he would spend hours helping to demonstrate in the laboratory to ensure that no student should make the mistake of preferring the word to observable fact. It was here that an episode occurred which though trivial is typical and serves to illuminate his character and relations with staff and students. A demonstrator detected a slip over fact in the previous lecture, so he at once told his Professor and was thanked. The Professor then announced the mistake to the class, and then patiently went round the large class to see that every student understood.

Of his work as Master of College a member of Downing should speak, I would merely mention the delightful evening at-homes to which large numbers continually came. The undergraduates of my own year who belonged to his College had the warmest friendship and deep respect for their Master.

Among his hobbies may be mentioned walking (and I believe in his younger days boxing) and architecture, especially the ecclesiastical, on which he wrote a number of short papers. One recognizes here too the same interest in form and in his particularly happy studies of the unique floral carvings on the pillars of Southwell Cathedral he was engaged on material which was in a curious way allied to fossil plants.

His general writings reveal him as a teacher, taking this word in its widest sense. He had no use for science which is the possession of a select few but believed in and worked for its dissemination. He spared no effort in popular lecturing, whether at large gatherings, like the Presidential meetings of the British Association

or at small local natural history societies. Very many will recall his rich and beautiful voice and will sadly regret that they can never hear it again.

Recognition of Seward's ability brought manifold extra duties. Many are the scientific committees and learned societies on whose councils he served, being uniquely fitted by his ripe judgement, energy and simple friendliness. Fitly they returned his help by giving him their highest honours; he was twice President of the Botanical section of the British Association, President of the Geological Society (from which he received the Wollaston Medal), and President of the Fifth International Botanical Congress. While still a young man he was elected a Fellow of the Royal Society, later serving on the Council, as Vice-President, Foreign Secretary, and receiving the Darwin and Royal Medals.

Truly Seward's was a rich and splendid life.

T. M. HARRIS

FORTIELLA SPHERICA SP.NOV., A NEW BRITISH ALGA

By DONALD H. MAGGS
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(With 5 figures in the text)

Of fresh-water algae *Fortiella* is a rare genus somewhat similar to *Chlamydomonas*. It resembles *Carteria* in having four flagella, and *Coccomonas* in having a rigid wall. There is one previous record of the genus in Europe, and there are two little-known Australian species (Pascher, 1927, p. 474).

The present species *Fortiella spherica* was found in an experimental tub behind the Botanical Department of Reading University. Some time before, the water in the tub had been manured with whey from sour milk, and it was consequently rich in organic matter. *Fortiella* was first found in mid-February when the water was covered with ice; it occurred in great numbers together with *Chlamydomonas* and saprophytic flagellates. When discovered, its reproduction had apparently ceased and the resting spores were being formed; attempts to stimulate it to active growth and division by adding more whey failed. As a consequence it has not been possible, so far, to work out the complete life cycle, and only the stages subsequent to the liberation of the daughter cells are described.

Diagnosis of *Fortiella spherica* sp.nov.

Lorica ferme globosa, 14μ–16μ diametro, verrucosa fusce. Flagella 25μ–30μ longitudine. Chloroplastum forma cortinae. Stigma parvum, anterolatere. Pyrenoidum absens. Amylum granulis globosis, multis sed dissipatis.

Description

A mature individual is shown in Fig. 2. The shell is brown and rigid enough to crack when pressed under a cover-slip. It is usually spherical or it may occasionally bulge to one side; but it is never flattened as in the closely allied genus *Pedinopera*, nor is the shell grooved or otherwise suggesting that it is made of two valves.

Some tests were carried out to ascertain the composition of the wall. It appears to consist of two materials, (1) a carbohydrate membrane impregnated with (2) a substance whose properties suggest it is hydrated ferric oxide, and to which is due the brown colour and rigidity of the shell. On treatment with potassium ferrocyanide followed by dilute hydrochloric acid, the shell turns blue, indicating the presence of a ferric compound.

Treatment with dilute hydrochloric acid alone, or with oxalic acid causes the

ferric oxide to dissolve away, and the wall becomes colourless and smooth; acetic acid, on the other hand, does not attack it. Supporting evidence that the shell is impregnated with an iron compound was provided by growing the organism in ferric citrate for a week when a further deposition of brown material on the wall occurred. The soft carbohydrate wall remaining when the ferric oxide is dissolved away may not be a true cellulose, since it does not stain at all with iodine in zinc chloride, nor with iodine followed by 70% sulphuric acid, but it is soluble in concentrated sulphuric acid. In no case did the addition of dilute acid even to a centrifuged mass of cells cause the evolution of a gas, so that calcium carbonate cannot be present in

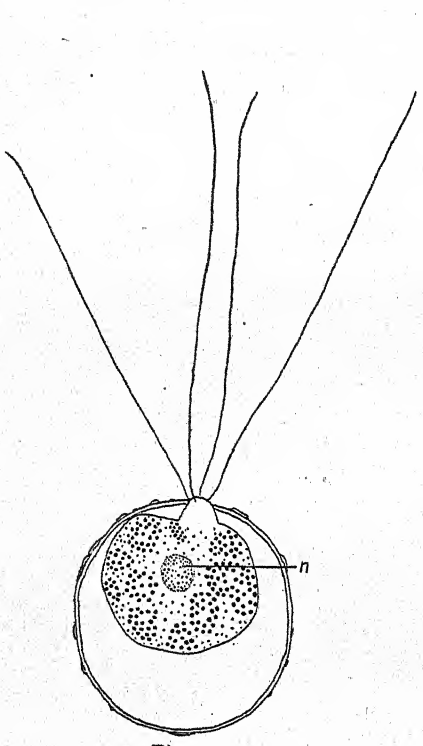

Fig. 1.

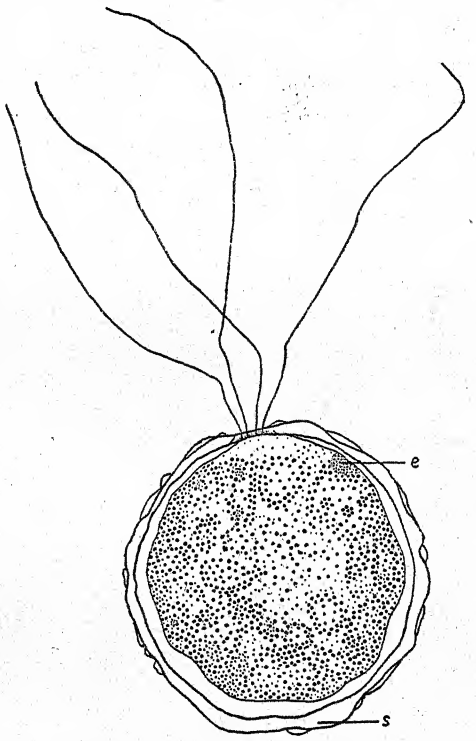

Fig. 2.

Fig. 1. Starved specimen, with shrunken protoplasm. *n*, nucleus. ×2500.
Fig. 2. A typical mature specimen, with flagella emerging from the pore. *e*, eyespot; *s*, shell. ×2500.

the wall. Pascher's statement in his description of the genus, that the wall is apparently strongly calcified, is thus not general.

The shell varies in diameter from 12 to 20μ, most specimens being 14–16μ in diameter.

The protoplasm is naked inside the shell, which it does not completely fill, and shows no separable membrane when shrunk in glycerin. The four flagella are attached at the pore to a colourless region of protoplasm, the papilla. They are 25–30μ long, and pull the alga along on a rather wobbly course without rotation. Compared with other organisms of similar size, its speed is rather slow, but it is

quite active enough to be abundant even in the surface water. Chloroplast, eyespot, nucleus and two contractile vacuoles can be distinguished. The chloroplast is large and shaped like a cauldron, the mouth towards the pore. There is no pyrenoid, and the chloroplast is loaded with scattered, round starch grains, not more than 4μ diameter, which always obscure the other cell structures. Occasionally the chloroplast was deeply lobed as if the protoplasm were beginning to divide. Several such specimens were watched in hanging drop cultures, but none of them divided, and those watched long enough eventually formed normal resting spores. The eyespot (Fig. 3) is red, oval and anterolaterally placed. It is frequently indistinguishable,

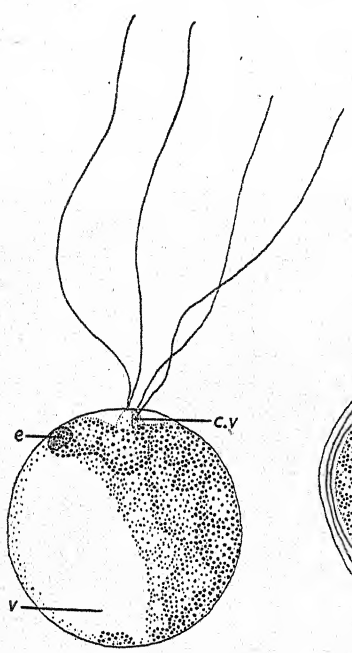

Fig. 3.

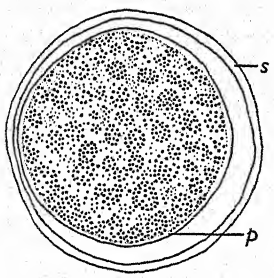

Fig. 4.

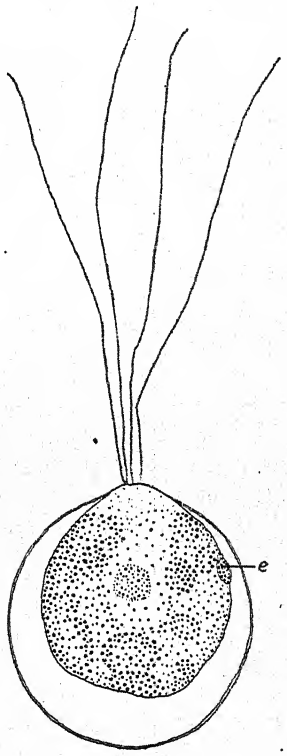

Fig. 5.

Fig. 3. Youngest stage found. The protoplasm completely fills the membrane. *cv*, contractile vacuole; *e*, eyespot; *v*, the large posterior vacuole. ×2500.
Fig. 4. Resting spore. *p*, the thin layer of colourless protoplasm surrounding the rest; *s*, the shell, which is now smooth. ×2500.
Fig. 5. Young stage shortly after loss of the posterior vacuole. The wall has just begun to thicken. *e*, eyespot. ×2500.

but may become more easily distinguished when specimens are mounted in dilute acetic acid.

The nucleus is also difficult to see, but staining in aqueous cotton blue after bleaching in alcohol showed it as a more deeply stained body. The contractile vacuoles are only shown in Fig. 3.

A specimen from a culture which was put in the dark and starved for a week is

shown in Fig. 1. The protoplasm has shrunk considerably, but a few starch grains still remain, although many similar cells in the culture were dying. The nucleus and papilla are more obvious than in the normal state.

As has already been said, division had apparently ceased when the alga was found, nor was it ever seen in hanging-drop cultures. However, a series of stages was seen which indicated the probable course of development from a young daughter cell to the mature individual and on to the resting spore. As the development was slow, the sequence was not observed for any single cell. In *F. brunnea* division is described as longitudinal by Pascher (1927, p. 474).

The youngest stage found (Fig. 3) is spherical, and is as big as a typical mature cell. On plasmolysis it is seen to be surrounded by a very delicate clear wall, which is completely filled by the protoplasm in the living cell. The chloroplast is saucer-shaped and placed rather on one side of the protoplast; and as it contains few or no starch grains, the eyespot and contractile vacuoles are more easily seen than in mature cells. There is also a large clear vacuole lying away from the papilla. This vacuole is a structure not described at all for the other species of the genus.

As development proceeds the delicate wall becomes thicker. Next the large clear vacuole disappears, and the protoplasm shrinks away from the wall all round except where the flagella emerge (Fig. 5). As a result the wall can now easily be seen.

It is possible that this phase when the vacuole is being lost may be of short duration, as no stages were seen in which the vacuole was intermediate in size. The chloroplast is by now larger and more granular. Then the wall gradually becomes yellowish and acquires the characteristic warts of the adult individual by the external deposition of the ferric oxide. Concurrent with this thickening of the walls the protoplasm slowly enlarges again, this time by increase in quantity, not by the formation of a new vacuole, but it never completely fills the shell. It is loaded with starch grains.

Early in March resting spores were of frequent occurrence, and after 12 March the motile stage was never found even in centrifuged concentrates. As the resting condition was assumed, the flagella were lost and the alga sank to the bottom of the tub. The chloroplast was packed with starch and usually the protoplasm nearly filled the shells, although some specimens were found where the volume of protoplasm was little more than half that of the shell. Later the wall becomes dark, grey and smooth (Fig. 4). It now gives a blue colour with potassium ferricyanide and not with ferrocyanide, so that it is now impregnated with a ferrous compound, probably ferrous sulphide, the ferric oxide having been reduced under the anaerobic conditions at the bottom of the tub. The grey colour disappears in a few minutes in air, and specimens kept in an open Petri dish were still brown at this stage. The protoplasm shrinks right away from the wall and a layer of clear protoplasm forms around the rest.

Treatment with glycerin does not cause a membrane to separate from the shrunken protoplasm. The eyespot and plastid retain their colour. Such stages stain slowly even in strong iodine solution, so that the shell or outer protoplasm must be rather impervious, but drying still causes shrinkage and disorganization of the protoplasm. So far the germination of these spores has not been observed.

Comparison

The designation of this alga as a new species seems justifiable on the following grounds. *F. spherica* differs from the European *F. brunnea* in the spherical and not long-oval shape of its shell, and in lacking a pyrenoid. It differs from the two Australian species, the shells only of which are described, in its shell being rounded, not, like *F. bullina* (Playfair) Pascher, emarginate in front, and unlike *F. scrobiculata* (Playfair) Pascher in being without pits in its shell. Like *F. brunnea*, *F. spherica* occurs in water rich in organic matter. Although its life history is incompletely known, the present account has established some new facts about the development and composition of the wall of this little known genus.

Summary

1. A new species of *Fortiella*, *F. spherica*, is described.

2. An interesting stage in the development of the daughter cells is described, when a large vacuole disappears and the protoplasm contracts away from the immature wall.

3. Information is given about the composition and the development of the wall.

Finally, I would like to thank Prof. T. M. Harris very much for drawing my attention to the alga, and both him and Dr Coulson for their encouragement and stimulating criticism.

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ON SOME PHASES IN THE LIFE HISTORY OF THE TERRESTRIAL ALGA, *FRITSCHIELLA TUBEROSA* IYENG., AND ITS AUTECOLOGY

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(With 49 figures in the text)

THE author, while engaged in the investigation of the soil complex of the 'Usar' land soils of northern India, came across this remarkable genus of the Chaetophoraceae recently established and described by Iyengar (1932) from south India and lately reported by Randhawa (1939) from fields lying fallow in the Fyzabad district. In view of its purely terrestrial habit it was thought advisable to work out its autecology, especially with respect to its soil requirements. Some observations on the physiological behaviour (the moisture relations) of the alga have been studied, together with considerable data about its life history.

After the first three or four showers of rain, the alga was found growing more or less gregariously in dark green clusters near the village of Pahari in an extended tract of 'Parti' or 'Usar' land at a distance of about 4 or 5 miles from the Benares Hindu University premises. It was collected for the first time in the beginning of July last. Since then it has also been obtained from an extensive alkaline land by the side of the road leading from the University to Chunar and a little farther from Akhari ponds. Iyengar (1932), however, reported the plant growing on moist silt of drying rain-water pools at Madras, as well as at Talguppa in the Mysore Province. Randhawa (1939) records its growth in a drying pond and on the banks of the river Sarju. It thus appears that the alga which has been found to grow on soils previously subjected to an extensive period of waterlogging, is now met with on such situations as are adversely characterized with regard to organic matter, nutrients, soil moisture and soil atmosphere. This ability of *Fritschiella* to invade and occupy alkali lands, the so-called virgin soils, therefore appears to be of considerable importance in connexion with the migration of plants from aquatic to terrestrial habitats.

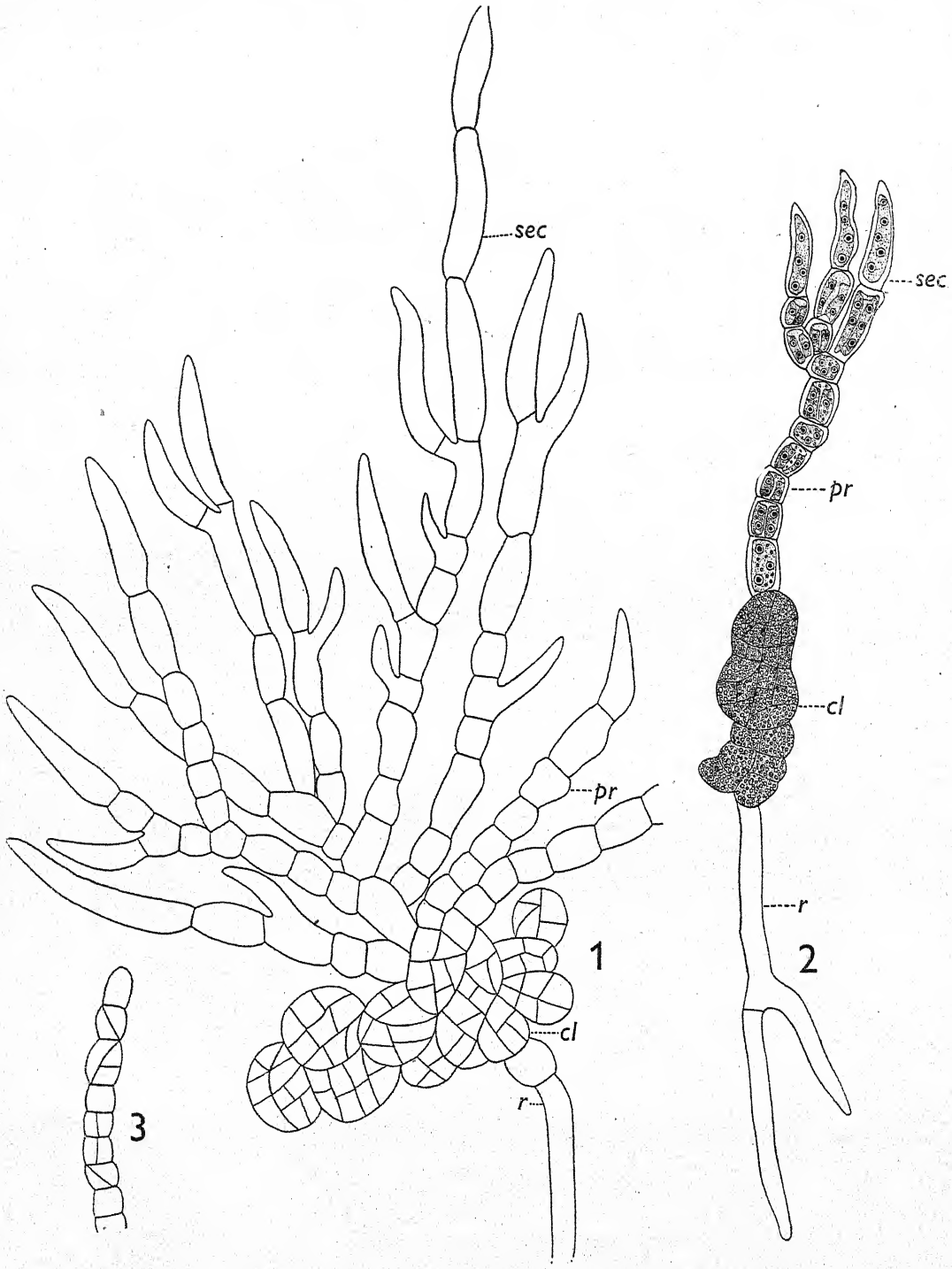
The mature plant exhibits four systems in its body. The first system, i.e. the *rhizoidal system*, consists of one or more downwardly directed rhizoid-like filaments, slightly branched (Figs. 1, 2), and composed of much elongated colourless cells, joined end to end, with very scanty contents, sometimes none. These rhizoids are always distinctly septate (Figs. 2, 10, 45, 46), both in young and mature plants, contrary to the statement of Randhawa (1939), that they are unseptate. The second system is the *prostrate system*, composed of a number of rounded or irregular swollen clusters of cells with thin walls, which become distinct only after the chlorophyll of their very dense contents is removed by treatment with alcohol, the

whole forming an irregular system with short congested branches (Figs. 1, 2, 4, 5, 10, 11). These cells are gorged with numerous starch grains of varying sizes and shapes (Fig. 2). The next system is the *primary projecting system*, which arises from the prostrate system and consists of a number of upright short-celled branched threads (Figs. 1, 2). It also contains cells with slightly granular contents. The fourth system is the *secondary projecting system*, composed of a tuft of slightly elongate branches, having longer cells with bright green contents (Figs. 1, 2). In the natural habitat, it is only this system that always arises above the surface of the soil.

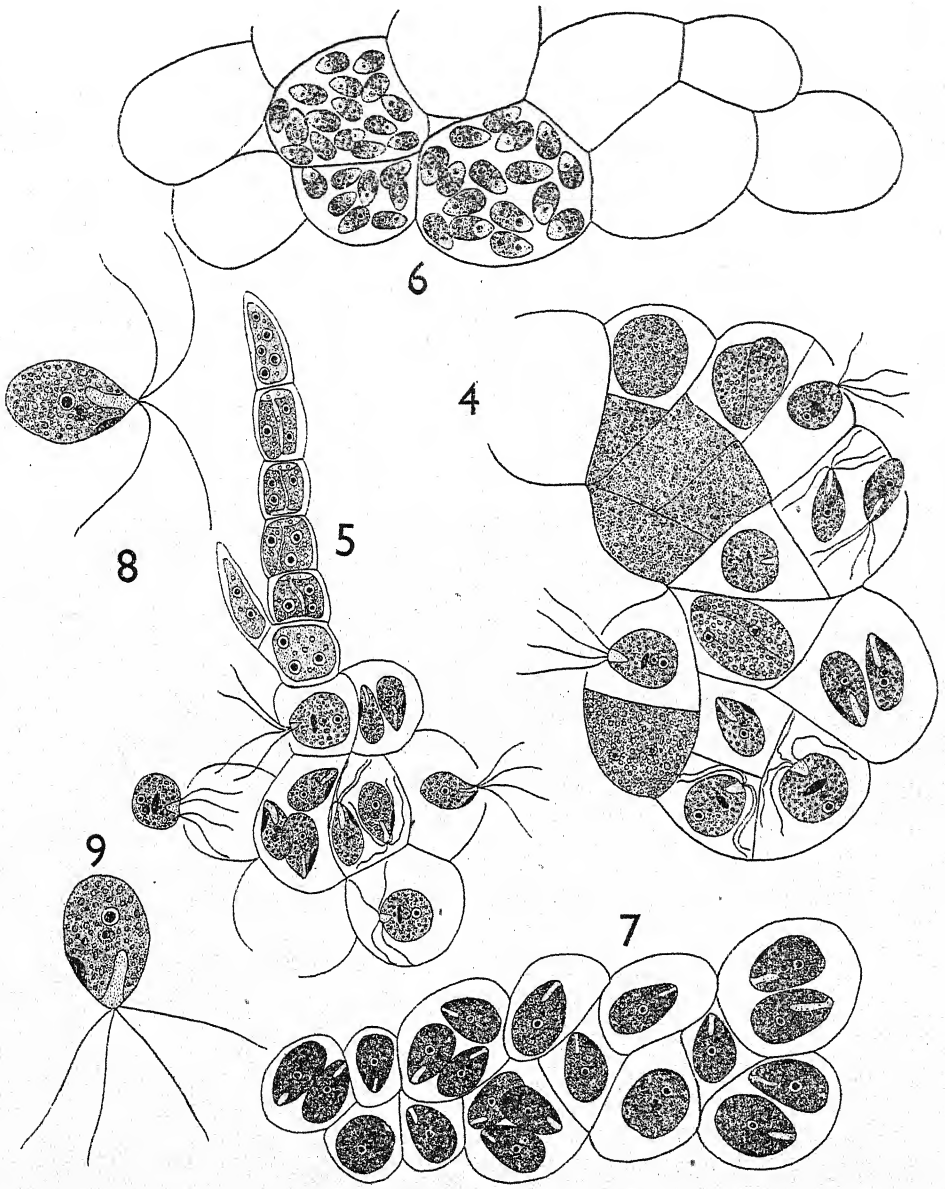
Swarmer formation and liberation

The formation of swarmers and their liberation has been studied in the material freshly collected from the fields as well as in that maintained in natural cultures in the laboratory. Spore formation is confined to the prostrate system of the plant, where each cell produces from one to many swarmers. The first indication of the process is afforded by a slight rounding off of the protoplast and its subsequent divisions in cases where two or more swarmers are formed (Figs. 4, 5, 11). The first division is always longitudinal (Fig. 4), and when only two swarmers are formed in a cell, these are oriented inversely (Figs. 4, 5). They have been seen to be liberated through a small lateral aperture in the thin cell wall. Liberation begins at 6 a.m. and lasts till 7 p.m., but the most active period is between 5 and 7 in the evening.

Three types of swarmers, like those found in the genus *Ulothrix* (especially in *U. zonata*), have been recognized. They are: the quadriflagellate macrozoospores formed singly in each mother cell (Figs. 4, 5, 7); the quadri- or biflagellate microzoospores, two or four in each cell (Figs. 4, 5, 7); and the biflagellate gametes that are formed in large numbers (Fig. 6), and of which the flagella could only be observed distinctly after liberation. The first two kinds of swarmers are formed simultaneously on the same plant (Figs. 4, 5, 7), but the last type is produced on different plants. The deep green macrozoospores (Figs. 20–23) are almost spherical or pear-shaped in outline and measure from 12 to 13·8 μ in diameter. They are gorged with numerous starch grains of varying sizes and shapes. The four equal flagella arise from a colourless protoplasmic papilla anteriorly. The stigma is flattened and median, and a bit nearer to the anterior end (Fig. 20). The more or less basin-shaped chloroplast possesses a pyrenoid almost close to the posterior end (Fig. 20). The nucleus is situated somewhere between the stigma and the pyrenoid (Fig. 20). The microzoospores (Figs. 8, 9, 24–31) are narrowly ovoid or fusiform. While the anterior side abruptly ends in a point, the posterior one is more or less rounded. Sometimes, however, both the ends are narrowly pointed (Fig. 28). The flagella, either four (Figs. 8, 9) or two (Figs. 27, 29) in number, arise anteriorly. Like the macrozoospores, they are also deep green in colour and gorged with numerous starch grains. They measure from 5 to 7·7 μ in breadth and 9 to 13·8 μ in length. There is a basin-shaped chloroplast which is deeply notched and possesses a single pyrenoid placed more or less medianly (Fig. 24). The stigma is situated



Figs. 1–3. *Fritschiella tuberosa* Iyeng. 1, a small mature plant with a single rhizoid. 2, a 15-days-old plant with a single septate rhizoid. 3, a portion of the primary projecting system showing longitudinal and diagonal divisions. *cl*, cluster of cells of the prostrate system; *r*, rhizoid; *pr*, primary projecting branches; *sec*, secondary projecting branches. All × 900.



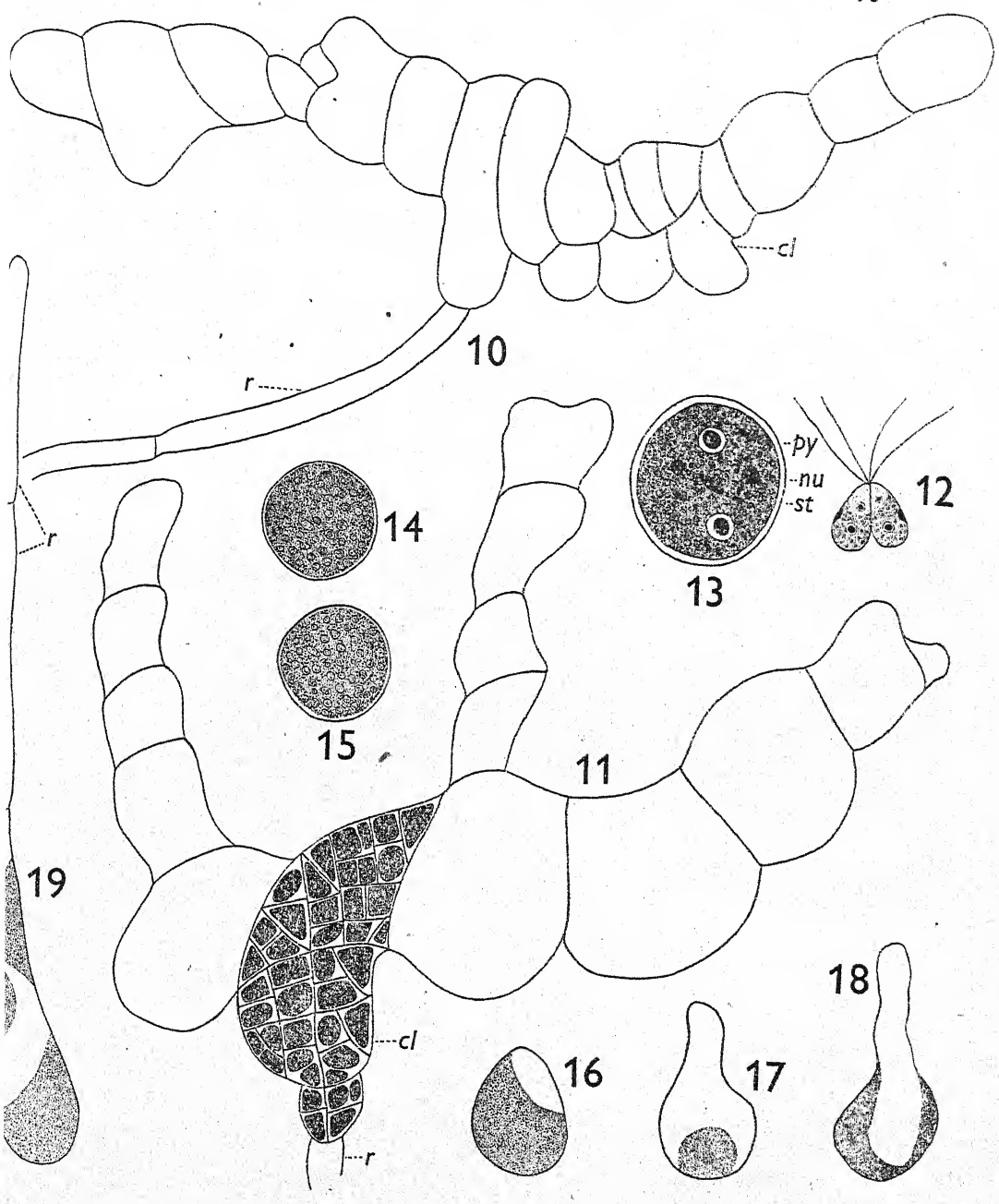
Figs. 4–9. *Fritschiella tuberosa* Iyeng. 4, a portion of the prostrate system, showing the formation of macro- and microzoospores. 5, a portion of the plant, showing the liberation of zoospores in living condition. 6, a portion of the prostrate system, showing the gametes, 7, a portion of the prostrate system, showing a number of zoospores with dense contents, which have escaped liberation. 8, 9, microzoospores. 4, 6, 7, × 1900; 5, × 1250; 8, 9, × 3200.

near the anterior end and forms a projecting ridge (Fig. 24). A single nucleus is present slightly above the pyrenoid (Fig. 24). Both the macro- and microzoospores exhibit positive phototaxy, and while moving forward they also rotate on their axes. The more or less fusiform gametes (Figs. 33–36) are almost of the same size. They possess two long flagella attached anteriorly and occasionally somewhat subterminally, and measure from 3 to 3·8 μ in breadth and 6 to 7·5 μ in length. The chloroplast with a single pyrenoid occupies almost two-thirds of the posterior region (Fig. 35). The linear eyespot lies laterally at the posterior end, and the single nucleus is placed more or less centrally in the hyaline anterior portion (Fig. 35). Their discharge takes place between 6 and 8 a.m. As soon as they are liberated, the gametes swim actively and swarm towards the illuminated side of the dish, where they continue to move for a period from a few to nearly 12 hr. Sooner or later the gametes from different plants meet in pairs and attach to one another by their anterior ends. The fusing gametes (Fig. 12) usually remain motile for a short time. They then lose their flagella, and gradually round off to form a zygospore which may at this stage show two chloroplasts, two pyrenoids, and two eyespots derived from the pairing gametes (Fig. 13). The zygote soon assumes its normal appearance and finally surrounds itself with a delicate wall. There is thus a marked similarity between these three types of swarmers and those of the genus *Ulothrix*.

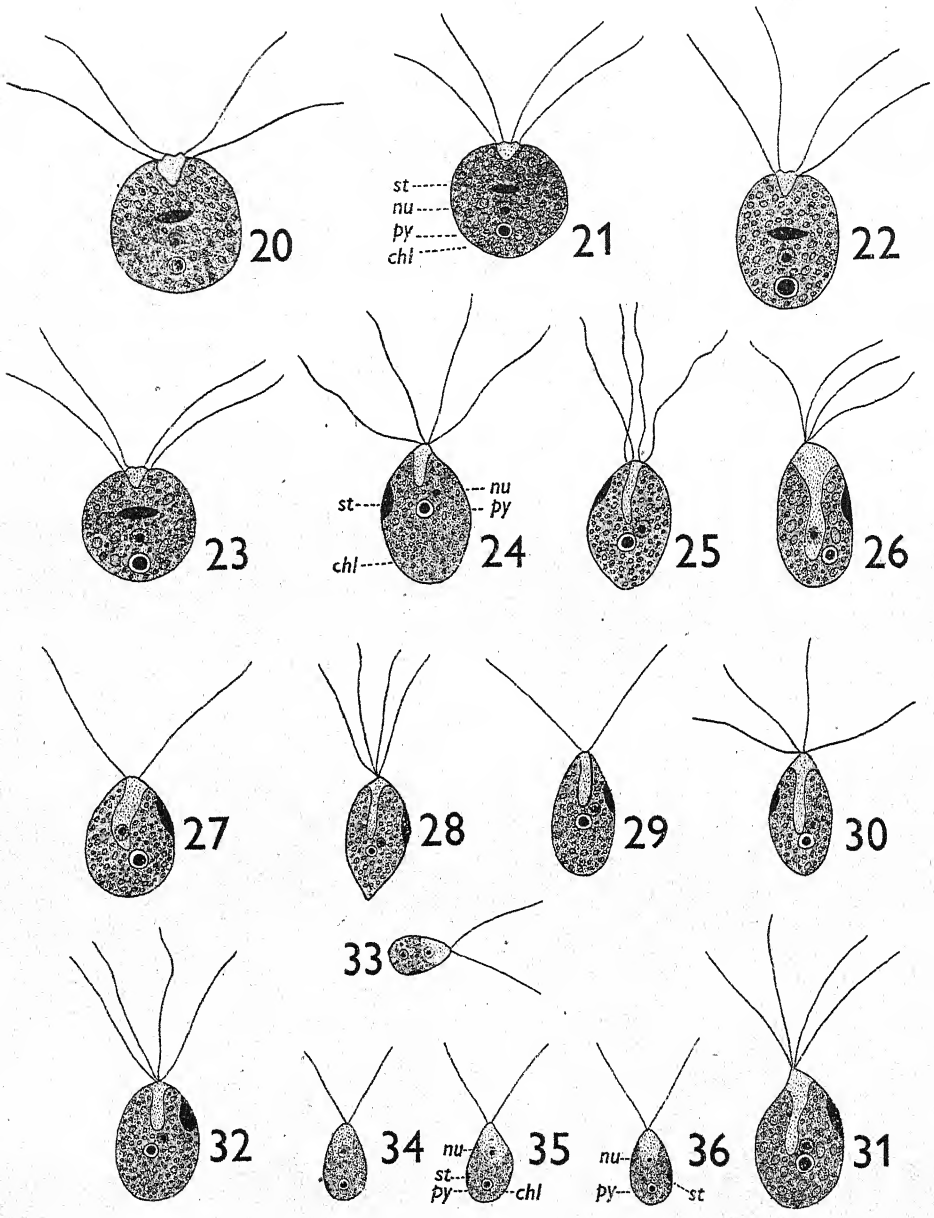
GERMINATION OF THE ZOOSPORES

After exhibiting movements, the period of which may not exceed more than 12 hr., the macro- and microzoospores become negatively phototactic and settle down on the side of the culture dish away from the light, where they become attached by their anterior ends. The flagella are then lost, and the cell rounds off and gets enclosed by a delicate wall (Fig. 37 *a–d*). On becoming ovate (Fig. 38) both kinds of zoospores begin to form at one end a hyaline protuberance which gradually grows in length (Figs. 39–42). It attains a considerable size and then cuts off a long rhizoidal cell by the formation of a transverse wall near its base (Fig. 43). This cell, which is devoid of all contents, grows much longer in course of time and gets divided by transverse walls into a two- or three-celled rhizoid (Figs. 44–46). It remains unbranched till after the development of a complete plant (Fig. 2). While the formation of the rhizoid is still proceeding at one end of the germinating spore another protuberance is given off at the opposite end (Fig. 45). Sometimes, however, both the protuberances are given out almost simultaneously (Fig. 40), but it is always the rhizoidal one that is the first to appear. The second protuberance also elongates and eventually becomes septate to form a linear row of four cells (Fig. 47), which become gradually wider from base to apex and possess rich contents. At this stage the whole young plant appears as an unbranched erect filament of six or seven cells and almost buried in the substratum. The later stages in the development of the mature plant are similar to those described by Iyengar (1932).

Each of the upper four cells of the young plant enlarges and sooner or later divides into four by transverse walls. Subsequently longitudinal and diagonal



Figs. 10–19. *Fritschiella tuberosa* Iyeng. 10, a plant with only the rhizoidal and prostrate systems of clustered cells developed. 11, the same as in 10, showing a stage in the formation of zoospores. 12, the fusing isogametes. 13, the newly-formed zygospore. 14, 15, zygospores which have not germinated. 16–19, stages during the germination of zygospore. *cl*, clusters of cells belonging to the prostrate system; *r*, rhizoid; *py*, pyrenoid; *st*, stigma; *nu*, nucleus. 10, 11, ×1250; 13, ×4000; the rest, ×3200.



Figs. 20–36. *Fritschiella tuberosa* Iyeng. 20–23, macrozoospores. 24–26, 28, 30–32, quadriflagellate microzoospores. 27, 29, biflagellate microzoospores. 33–36, gametes. *chl*, chloroplast; *py*, pyrenoid; *st*, stigma; *nu*, nucleus. All ×3200.

divisions occur (Fig. 48), resulting in the formation of daughter cells in all directions, though mainly along planes at right angles to one another. Ultimately an irregular group of rounded cell clusters is formed, each cluster being the result of the repeated divisions of one of the four original cells or one of their products (Fig. 49). In many of these clusters localized growth occurs, so that new clusters are budded out laterally or the whole original cluster may produce lobes, from the end-cells of which long septate rhizoids (4·5–8·3 μ in breadth) grow out, there being only one rhizoid coming out from such a cell.

The above-mentioned rounded clusters (about 20–45 μ in diameter) of cells represent the prostrate system of the alga. From this system, especially from the uppermost clusters, a number of filaments arise and grow upwards. They are generally composed of short iso-diametric cells (6–10 μ broad and 5–10 μ long) with dense contents, and constitute the primary projecting system. Cell division in these threads takes place usually transversely, though occasional longitudinal, cross-wise, or diagonal divisions may occur (Fig. 3). Rounded clusters, like those seen in the prostrate system, are never formed. The first branches of this projecting system show considerable ramifications directed upwards. From the cells of these ramifications there arise a number of secondary branches (secondary projecting system) the cells (6–10 μ broad and 3–8 times as long as broad) of which are much elongated and possess comparatively scanty contents. These cells, with the exception of the terminal ones, generally grow out laterally to form short branches which usually consist of only one cell with a broad conical apex. Sometimes, however, no septum is formed to cut off this one-celled branch from its parent cell (Fig. 1). There is no production of any hairs whatsoever.

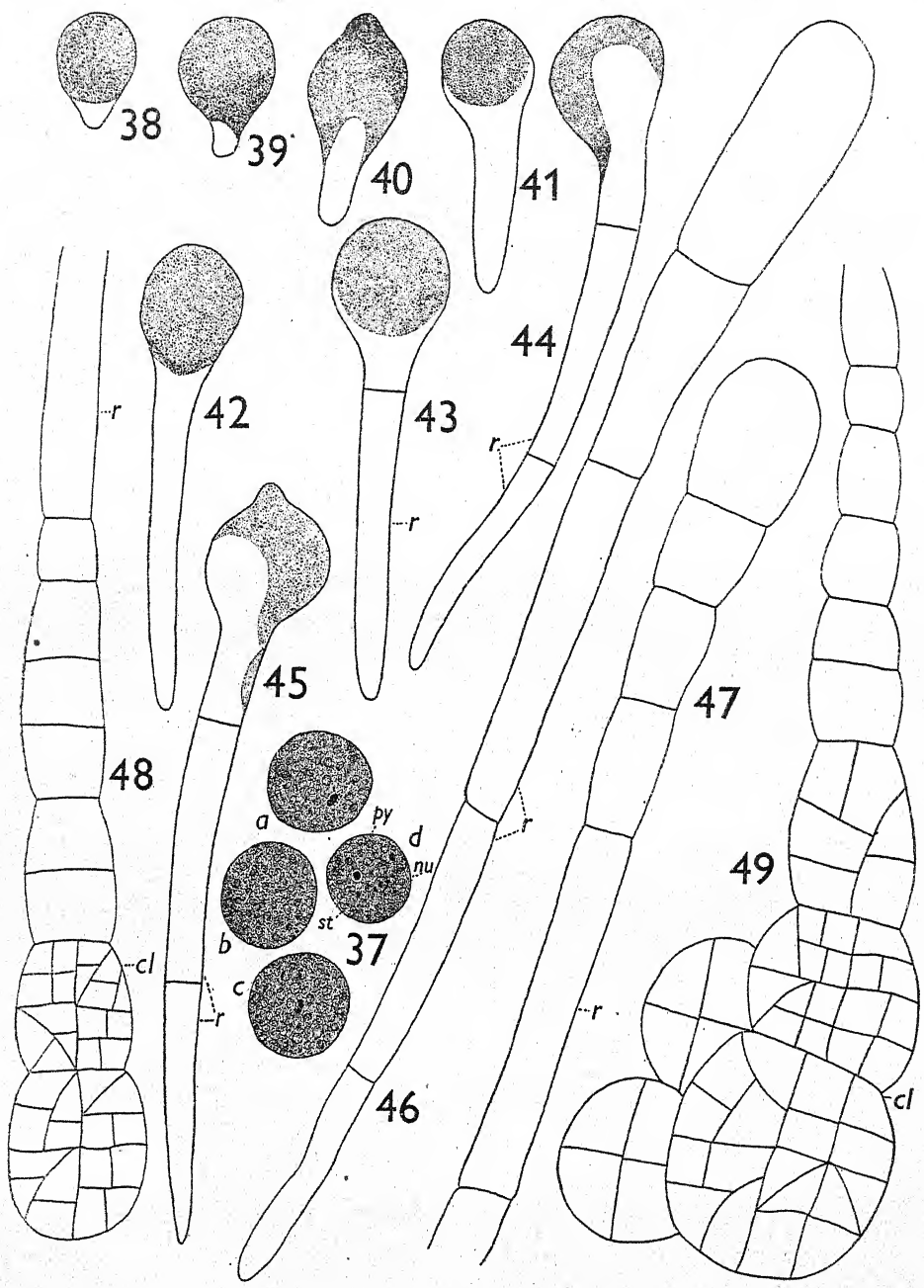
A curved plate-shaped chloroplast, with two to eight pyrenoids, is distinctly seen in the cells of the primary and secondary projecting systems (Fig. 2). The contents of the cells of the prostrate system, and also occasionally of those of the primary projecting system are gorged with starch grains to such an extent that the chloroplasts are not clearly recognizable (Fig. 2). The pyrenoids in these cells are smaller and fewer in number than in those of the secondary projecting system. Each cell contains a single nucleus.

Germination of zygotes

The zygote does not take any rest and germinates directly into a new plant, the process being more or less the same as that for the swarmers (Figs. 16–19).

The moisture relations of the alga

In order to study the moisture relations of the alga, soil blocks with the normal growth of the plants were transported quickly to the laboratory. The plants thus obtained were treated immediately with different concentrations of a plasmolysing solution of sea salt (cf. Fritsch & Haines, 1923). The results thus obtained are summarized in Table 1.



Figs. 37–49. *Fritschiella tuberosa* Iyeng. 37, *a–c*, resting macrozoospores; *d*, resting microzoospore. 38–49, various stages in the development of the new plant. *py*, pyrenoid; *st*, stigma; *nu*, nucleus; *r*, rhizoid; *cl*, clusters of cells belonging to the prostrate system. All × 3200.

It may be noted from this table that the cells of the prostrate system require the highest concentration for complete plasmolysis. The cells in the primary projecting system get plasmolysed in 9–13 % of sea-salt solution, and those of the secondary projecting system in 3–5 % solution. The abnormal behaviour of the cells in the prostrate system appears to be due to the fact that they are gorged with numerous starch grains, a view that has already been expressed by Fritsch & Haines (1923). Moreover on the onset of the unfavourable conditions of desiccation the secondary projecting system is the first to disappear and then the primary projecting system. It is only the prostrate system that survives and perennates as such (cf. Iyengar, 1932). It is, therefore, concluded that in the cells of this system there is a marked retention of a considerable amount of moisture due to the high concentration of the sap.

Table 1. *The behaviour of cells of the alga to different strength of sea-salt solution*

Strength of solution %	Secondary projecting system		Primary projecting system		Prostrate system	
	Plasmolysed %	Unaffected %	Plasmolysed %	Unaffected %	Plasmolysed %	Unaffected %
1	40·5	59·5	2·3	97·7	—	—
3	93·7	6·3	12·5	87·5	—	—
5	100·0	—	22·8	77·2	—	—
7	100·0	—	52·3	47·7	—	—
9	100·0	—	97·4	2·6	—	—
13	100·0	—	100·0	—	23·5	76·5
17	100·0	—	100·0	—	78·2	21·8
21	100·0	—	100·0	—	100·0	—

Table 2. *The results of the analyses of the different soil samples*

Sample no.	Moisture content %	Organic content %	*p*H	E_h m.V.	E_7 m.V.	Thio-cyanate	NO_3
1	4·35	1·2	9·7	344	500	o/o	+
2	2·95	1·8	10·5	310	513	o/o	+
3	3·32	1·1	9·6	365	515	o/o	+
4	2·12	1·2	10·2	275	460	o/o	+
5	1·95	2·2	11·1	233	470	o/o	+
6	1·96	2·3	11·0	248	480	1/o	−
7	2·32	1·8	9·5	380	525	o/o	+
8	3·42	1·5	9·5	365	510	1/o	−
9	2·35	1·6	10·8	230	450	1/o	+
10	2·45	1·3	10·9	250	476	o/o	+
11	4·12	—	11·0	268	500	o/o	+
12	4·32	—	9·6	375	525	o/o	+

The edaphic complex determining growth of the alga

As has been mentioned previously, the alga is found to grow in the 'Usar' land soils, and a study of its soil requirements was therefore undertaken. Factors like moisture and organic contents, *p*H, E_h, exchangeable bases, and nitrates have been investigated, and the various results obtained are summarized in Table 2. The

methods employed in connexion with the study of these factors are those suggested by Emmert (1938), Wright (1939), Pearsall (1938), and Misra (1938). Soil samples characterizing the growth of the alga were taken from the fields and analysed the same day. Most of the observations were, as far as possible, made in nature.

From Table 2 it may be observed that the plant grows in soils having very low moisture and nitrate contents and also under extremely alkaline and oxidizing conditions. The fact that the alga flourishes with very insignificant organic content of the soil appears to be of considerable evolutionary importance.

DISCUSSION

Iyengar (1932) did not come across the motile stages of the alga and therefore he could not definitely decide about its systematic position. But, on account of its certain resemblances with *Iwanoffia*, *Stigeoclonium* and other members of the Chaetophoraceae he regarded it a member of this family.

The author's exhaustive study of this alga confirms the above observations of Iyengar. In possessing well-defined sexual and asexual methods of reproduction by swarmer formation, the alga resembles members of the subfamily Chaetophoreae. The formation of the three types of swarmers, viz. the quadriflagellate macrozoospores, quadri- or biflagellate microzoospores, and biflagellate gametes, brings *Fritschiella* quite close to the genus *Ulothrix* (especially *U. zonata*), as well as to the two species of *Stigeoclonium* (*Stigeoclonium longipilum* and *S. fasciculare*) studied by Pascher (cf. Fritsch, 1935, p. 255). In the production of large quadriflagellate macrozoospores it resembles some of the members of the Chaetophoreae. The formation of quadriflagellate microzoospores is in common with *Stigeoclonium* and *Draparnaldia* and that of the biflagellate ones with all the members of the subfamily. On account of these resemblances *Fritschiella* can safely be included in the Chaetophoreae. But its collar-shaped chloroplast, with 2–5 conspicuous pyrenoids, and three types of swarmers also make it closer to the Ulotrichales. It thus appears that *Fritschiella* has affinities with both the Chaetophorales and the Ulotrichales. This may be due to the probable common origin, along divergent lines, of both these orders (cf. Fritsch, 1935).

The formation of swarmers as previously stated is only confined to the prostrate system of the plant, and this makes true Iyengar's presumption that 'the thin walls of the individual cells of each cluster will facilitate the escape of motile spores, if such, as seems probable, should be formed in these cells when the rainy season commences'. Randhawa (1939) criticizes Iyengar's statement on the ground that the former found thick-walled cysts in his material, both free in the soil as well as intact with the plant, in the system in question. During the course of the study of the life history of the alga the writer has not come across any cysts, but he has found in the soil a number of thick-walled zygotes (Figs. 14, 15), and also rounded zoospores which have lost all motility (Fig. 37). They resemble to a very great extent the structures which Randhawa has described as cysts. He has worked on preserved material; and a careful examination of his figure (1939, Figs. 2, 6) shows

that there is a space between the wall and the cytoplasm, probably due to the shrinkage of the latter on account of the action of the preservative. His Fig. 2 (5), showing the conversion of cells of the prostrate system into cysts in a mature plant, also appears to the writer to be a stage in the process of swarmer-formation, which has abruptly stopped due to sudden loss of water by intense evaporation from the soil. In the light of the present investigations Randhawa's findings are doubtful and his criticism of Iyengar's statement untenable. It is probable that he has wrongly interpreted these bodies as cysts because of the fact that he has not studied their further development.

In tracing back the origin of a land flora, Bower (1935) attaches great significance to *Fritschiella*. He says (p. 498): 'Its interest lies in the terrestrial habit, and in the fact that this amphibial plant possesses the essentials of a three-dimensional, photosynthetic sub-aerial thallus originating from a simple filament.' The writer's observations support this view. The autecological behaviour of the alga is rather peculiar. Its invasion and encroachment upon the 'Usar' land soils of Northern India is a fact of considerable evolutionary importance. These soils, as stated before, have been found to be the so-called virgin soils, which are adversely characterized with regard to soil moisture, organic content, and nutrients. The alga grows with a highly alkaline condition of the medium with the *p*H values ranging from 9·5 to 11·1, and the redox potential varying between 450 and 525 m.V. (E_7 values). The fact that the alga grows on soils having very low moisture and low organic content is interesting. It shows therefore that *Fritschiella* might have been one of the colonizers and survivors out of the pioneers which played a great rôle in the conquest of land when the great landward movement took place in the remote past of the earth's history.

The initial developmental stages of *Fritschiella* resemble to a great extent those of the gametophyte of *Equisetum debile* Roxb., described by Kashyap (1914, cf. Figs. 1–15). The starch-gorged cells of the prothalli of certain ferns look very similar to the cells of the prostrate system of the alga. In early stages this alga also resembles the prothalli of *Schizaea*. Very probably the first land plants originated and evolved from humble ancestors like *Fritschiella*, and not from massive sea-weeds which were already too specialized and over-developed, and lacked the plasticity which alone could have ensured success in a new environment.

Summary

The life history of the terrestrial alga *Fritschiella tuberosa* Iyeng. has been studied. Three kinds of swarmers, the quadriflagellate macrozoospores, the quadri- or biflagellate microzoospores, and the biflagellate gametes, like those we find in the genus *Ulothrix*, are formed. The swarmer formation is confined to only the prostrate system of the plant. Similar gametes from different plants fuse together to form zygotes, which germinate directly to give rise to new plants.

The autecology of the alga has been studied. The plants grow under highly alkaline and oxidizing conditions of the medium. The *p*H value ranges from 9·5 to

11·1. The redox potential has been found to vary between 450 and 525 m.V. (E_7 values). The ammonium thiocyanate test is without any result and the diphenylamine test is positive though not appreciable. The organic content of the soil is more or less negligible (1–2·3 %), and the moisture content is also very low.

It has been found that the cells of the different systems of the plant behave differently with regard to the different solutions of sea salt. The cells in the prostrate system of the plant require highest strengths for complete plasmolysis.

Perennation is brought about by the prostrate system of the plant, the cells of which remain as such in the soil during the dry period. They are able to do so because they are densely gorged with starch grains.

The evolutionary significance of the alga has been discussed, and its inclusion in the family Chaetophoraceae is established.

In conclusion, I have much pleasure in expressing my great indebtedness to Prof. Y. Bharadwaja for his kind guidance and criticism throughout the course of this investigation.

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THE FORMATION OF 'H-PIECES' IN THE WALLS OF *ULOTHRIX* AND *HORMIDIUM*

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(With 3 figures in the text)

THERE would appear to be little information upon any marked thickening in the walls of vegetative cells of *Ulothrix* and *Hormidium*, or of the formation of H-pieces, comparable with those found in species of *Microspora*. West (1916) hints at their occurrence in *Ulothrix zonata* (cf. his Fig. 181) in connexion with aplanospore formation, while Fig. 53 F and G of West & Fritsch (1927) clearly indicate that they have been observed in *Ulothrix aequalis*, both during aplanospore development and in the liberation of macrozoospores. Smith (1933) goes so far as to state, 'All genera referred to the Ulotrichaceae have a cell-wall that is not composed of overlapping H-pieces', but this statement is probably a *lapsus calami*, for he implies as well that H-pieces occur in *Binuclearia* and *Radiofilum*, both of which genera he includes in this family. It is well known that the cell membrane of some Chlorophyceae consists of inner and outer parts, and sometimes includes a bounding cuticle. West (1916) states that most species of *Ulothrix* overwinter as short filaments with thick transverse and lateral walls, and that when active cell division starts, these old, thick walls are burst apart as the cells divide.

Piercy (1917), in her investigation of *Hormidium*, concluded that splitting of the filaments was due to separation at the middle lamella, and implied that this layer may increase considerably prior to separation of adjacent cells, and even extend along the longitudinal walls from cell to cell. She also noted the presence of a cuticle in *H. flaccidum*, and deduced from inconclusive tests that this layer was mucilaginous, or at least that it became so, and not cutinized. Investigations of earlier workers on the separation of cells in this genus are referred to in her paper.

In the late winter of 1940 collections of *Ulothrix zonata*, from the River Ogwen, near Bangor, yielded some filaments in which the septa between adjacent cells were occasionally thickened, and sometimes laterally swollen, to a remarkable degree (Fig. 1): these thickenings immediately suggested a condition comparable to that obtaining in the walls of some species of *Microspora*, and probably to the somewhat elaborate walls of *Cladophora*. Each cell was surrounded by an inner

wall (Fig. 1 D, F, *iw*), in which stratification was often visible, while an outer layer of wall was often continuous along the filament (Fig. 1 F, *ow*) and across the septa (Fig. 1 D, *ow*): as far as could be ascertained there was a continuous, delicate pellicle external to the outer wall. The H-shaped swellings might have been produced either by the outer wall in the region of the septum, or by a swelling of the middle lamellae, the swelling being accompanied by lateral expansion which caused the walls to bulge in this region.

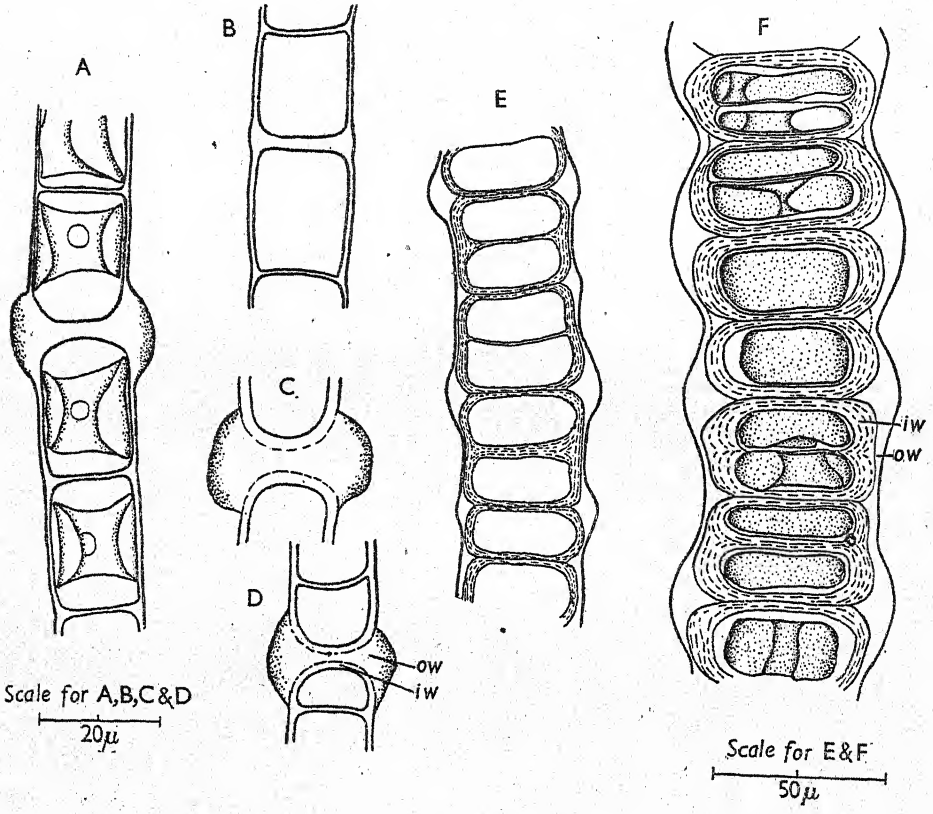

Fig. 1. *Ulothrix zonata* (Web. et Mohr) Kützt; pieces of filaments showing swollen cell walls. A and B were drawn from living material, C and D from material treated with ruthenium red, E from material stained in Congo red, F from material treated similarly to E, then mounted in dilute glycerine. *iw*, inner wall; *ow*, outer wall.

These swellings, which will be referred to as plugs, were subjected to microchemical tests. With Schultze's solution there was a doubt as to whether the plugs were very faintly coloured or not, but the test could certainly not be regarded as affording conclusive proof that they were composed of cellulose. Similarly the application of iodine followed by sulphuric acid led to a faint violet coloration, but this test likewise could hardly be regarded as conclusive. When immersed in ruthenium red the plugs sometimes assumed a very faint pink tinge after a lengthy period, and this may perhaps be regarded as indicative of the presence of traces

of pectin in these structures; again, however, it was impossible to regard this test as conclusive, and it may be added that the contents of adjacent cells were coloured by the dye. After treatment with 6% aqueous potash, both cold and warm, the plugs appeared as highly refractive bodies, without any trace of stratification.

When filaments were treated overnight with alcoholic potash, and subsequently tested for cellulose with iodine and sulphuric acid, a positive reaction was obtained in the plugs. Even after this potash treatment, however, no positive reaction for pectin was obtained with ruthenium red. These results indicate that there is a 'cuticle' or pellicle of a fatty nature bounding the filaments, and inhibiting the penetration of reagents into the walls; it might be expected that treatment with potash would render such a layer more easily penetrable. It may be added that filaments were allowed to soak in 5% hydrochloric acid overnight and then washed in water for 3 hr.: after this treatment it was possible to obtain a blue coloration of the plugs with methylene blue, a coloration which could not be obtained without such preliminary acid treatment.

These tests lead to the conclusion that the plugs are greatly swollen regions of the outer layers of the cell wall, and are not derived from a thickening of the middle lamella. At times (Fig. 1 C, D, E, F), the inner wall adjacent to the plugs is easily seen, this inner wall showing stratification.

Material of *Ulothrix zonata* was collected from the same locality in early May 1941; in this, very few swollen plugs were encountered, but the inner and outer layers of the wall—the former only, stratified—could easily be seen in the region of the septum. The presence of a pellicle of fatty material was indicated by positive reactions with Sudan blue and Sudan black. This pellicle varied somewhat in thickness; in parts it was well defined and continuous, elsewhere thinner; and sometimes not evenly distributed. Outside the pellicle was a thin layer of mucilage (Fig. 2 A). Filaments which had been allowed to dry out on a slide often had a moniliform appearance (Fig. 2 B), produced by the septa contracting to a greater degree than the cells themselves; it is concluded that the outer layers of the wall are more watery (probably more mucilaginous) than the inner wall, and shrink more upon drying, because they lose a greater amount of water. There is no trace of pectin in these outer layers (tested with ruthenium red). It is inferred that the outer layers (outer wall) are different, chemically, from the inner wall; they may well represent an intermediate stage in the degradation of cellulose to mucilage.[1] It is doubtful if the plugs or H-pieces were in this condition; their highly refractive appearance after treatment in potash seems to militate against such an assumption.

The presence of a thin layer of mucilage outside the pellicle is of interest; it is difficult to visualize the change of a fatty material, and the pellicle is undoubtedly of a fatty nature, into mucilage; equally, it does not seem likely that mucilage, formed from the outer surface of the outer wall, could pass through the impervious pellicle. It seems probable that the outer layers of the wall become mucilaginous before the formation of the fatty pellicle just within their boundary.

[1] There is a resemblance between these plugs and the cell structure at cell disjunction in certain species of *Spirogyra*, cf. Fritsch (1935).

There was no evidence that the swollen plugs functioned as separation disks, for the filaments did not seem to be more liable to break in such regions than elsewhere. The formation of such plugs is apparently rare in *Ulothrix*, for it is difficult to believe that they would not have been recorded, were it otherwise. It is, perhaps, not unreasonable to regard the condition as pathological, and it may be noted that the plugs occurred soon after the weather had been exceptionally cold for a protracted period.

During the early months of 1941, plugs of an appearance similar to those just described were found in a few filaments of a *Hormidium* collected in a pool at Criccieth. We refer the plant to *Hormidium flaccidum*. Unfortunately so little

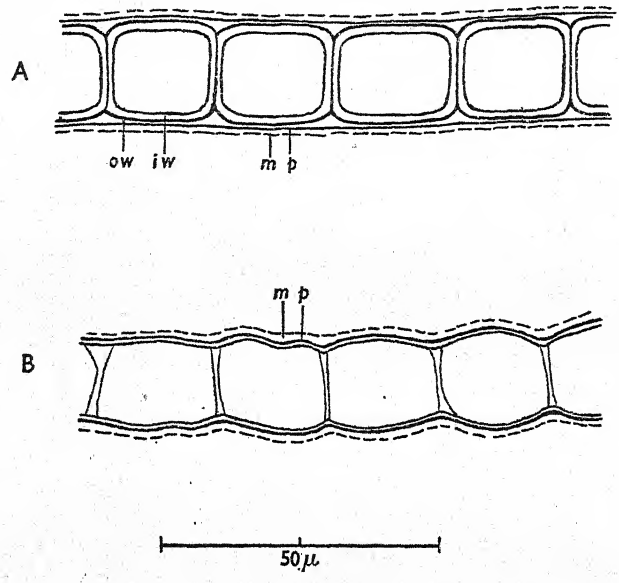

Fig. 2. *Ulothrix zonata* (Web. et Mohr) Küntz; A, part of a living filament; B, part of a filament which has been dried out on slide. *iw*, inner wall; *m*, mucilage; *ow*, outer wall; *p*, pellicle.

material was available that it was not possible to carry out any microchemical tests, but our observations leave little doubt that the plugs are similar in nature and origin to those described above in *Ulothrix zonata*. In the *Hormidium*, however, they are directly concerned with fragmentation of the filaments (Fig. 3 D, E, F) and become more extensive than those of *Ulothrix*, so that eventually the whole of the cell wall becomes thickened, at times more or less uniformly. Weak zones are developed approximately in the middle of the cell wall (Fig. 3 F, *w*); here the outer wall eventually breaks and usually becomes hinged back on itself, liberating the cell still enclosed in the inner wall. The cells so liberated proceed to divide and to form new filaments (Fig. 3 D, E, F). It may be that elongation of the cell is responsible for the rupture of the outer wall.

In some instances remains of the pellicle could be made out in the region of the septum (Fig. 3 A, B, D, E), but elsewhere, and especially in the cells of the

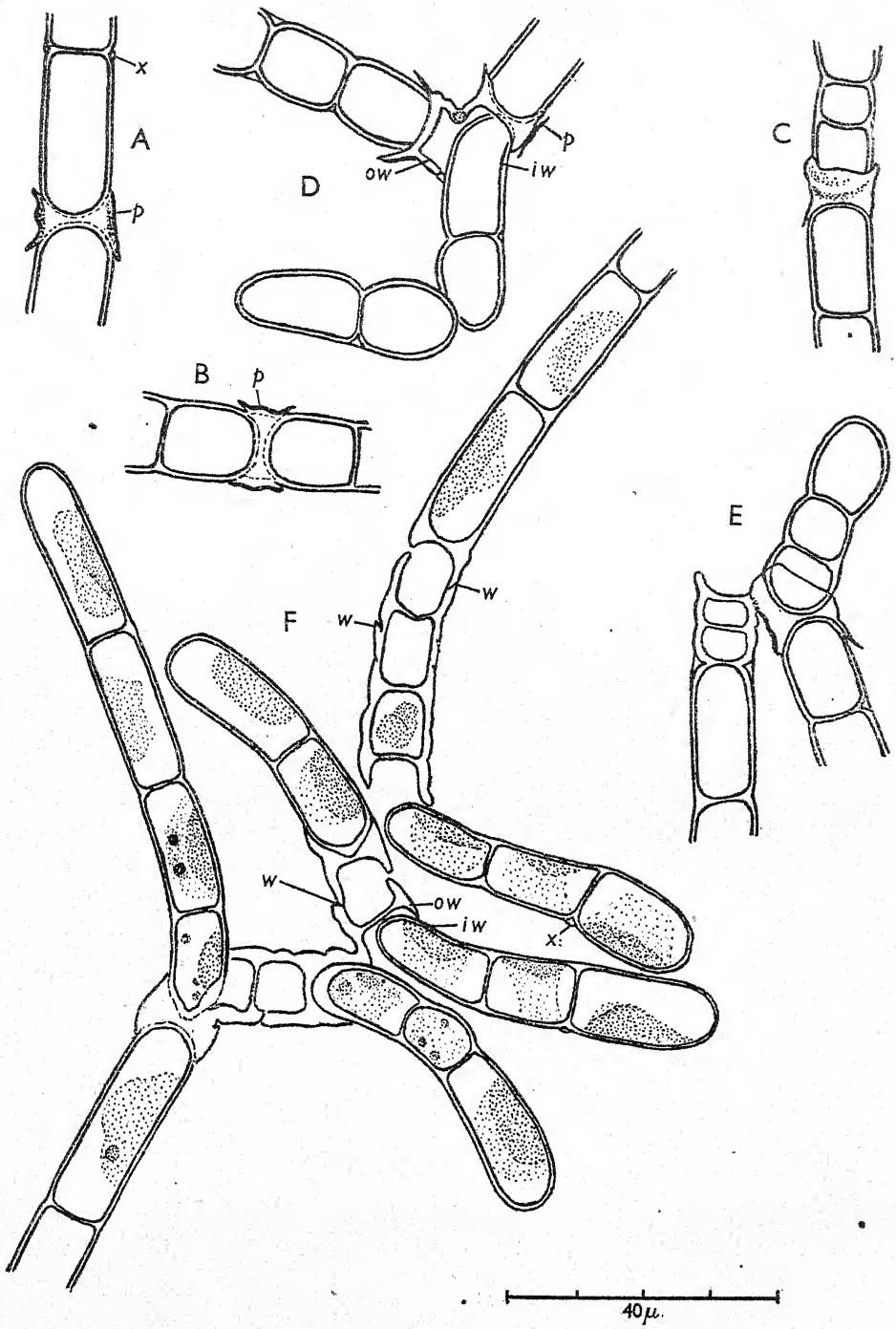

Fig. 3. *Hormidium* ref. *flaccidum* (Küntz) A. Br. A–F, parts of one filament showing 'H-pieces' and fragmentation. The scale of A and B is slightly greater than that shown for the remainder. Note beginnings of swellings at *x* in A and F. *iw*, inner wall; *ow*, outer wall; *p*, pellicle; *w*, zone of weakness.

newer filaments, no stratification of the wall, or even distinction between inner and outer wall layers, could be seen. This is due, at least in part, to the thinness of the walls, but perhaps also to the outer wall and pellicle not having developed.

The *Hormidium*, like the *Ulothrix*, was collected soon after a period of exceptionally cold weather, and it is possible that the marked development of the outer wall was conditioned by these abnormal conditions.

SUMMARY

1. Peculiar swellings of the septum and adjacent walls are described for *Ulothrix zonata* and *Hormidium* ref. *flaccidum*.

2. In the *Ulothrix* the wall consists of inner and outer portions, the latter bounded by a pellicle of a fatty nature.

3. Microchemical tests on the *Ulothrix* indicated that the swellings were produced by the outer cell wall. This is probably true of the *Hormidium*.

4. In the *Hormidium* these thickenings serve as a means of fragmentation of the filament, but in the *Ulothrix* no evidence exists that the filaments break more readily in the region of the thickened walls than elsewhere.

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VARIATION IN *VIOLA RIVINIANA* RCHB.

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(With Plate 2)

Introduction and summary

The aim of this paper is to present an account of variation in *Viola Riviniana* Rchb. The scope of the account is limited in several ways; for it is mainly confined to the species as it is known in the British Isles; and it is only preliminary, as the work has been in progress for a relatively short time.

The method has been as follows. The plant has been examined in the herbarium and in the field; families have been bred from selected examples, and crosses made between them; some cytological investigations have also been made. We have then viewed critically the existing taxonomy of the species, and our data have led us to propose that it should be modified.

This type of procedure has come to be known as The New Systematics (Huxley, 1940); and the model for such investigations must be, in our opinion, the important and admirable work of Gregor and his collaborators (1936, 1938, 1939) on *Plantago maritima* L. The present paper does not attempt the task on their scale, and is indeed only a preliminary clearing of the ground. Emphasis has been laid, for the time being, on the purely taxonomic aspect of the subject, in order that the results that have been obtained may become available at once. It is particularly hoped that they will be useful to ecologists.

The following are the main heads of the paper:

(1) Introduction and summary.
(2) Notes on method.
(3) Description of *Viola Riviniana.*
(4) Subspecies *nemorosa* and subspecies *minor* of *Viola Riviniana.*
(5) Variation in the subspecies of *Viola Riviniana*, especially subspecies *nemorosa.*
(6) Cytology.
(7) Taxonomy.
(8) Concluding remarks.
(9) References.

Method

Investigations were at first centred on the violets which were to be found in the ash-oak woods near Cambridge; and it is on these that most of the work has so far been done. Collections have, however, been made in many other parts of the country. The collections have been small, and the aim so far has been to get a large number of small samples rather than to take large samples of populations.

The plants have been potted, and grown in a cool greenhouse. Under such conditions they flourish and flower abundantly, and this facilitated work on inter- and intraspecific hybridization; collection of seed in the field presents difficulties, owing to the explosive mechanism of the violet fruit.

The available herbarium material of Great Britain has been fairly thoroughly studied, thanks to the courtesy of the directors of the various herbaria; the information so obtained has been compared with that obtained from the study of the living plants. It may be mentioned here that study of violets in the dried condition is not always easy; in particular, flower colours and patterns of petal variation, as well as shape and disposition of the petals, are difficult to determine. In addition, the facies of the violets varies greatly with the season of the year; and as the herbarium sheet has usually a gathering made at one season only, an additional difficulty is introduced. With the living plant, these difficulties are not troublesome.

DESCRIPTION OF *VIOLA RIVINIANA*

Viola Riviniana Rchb. has a short erect stem, with a terminal bud which produces a rosette of three or four leaves. In the axils of these leaves are borne leafy flowering branches; but the terminal bud does not lengthen into a flowering branch. Other British violets which share these characters are *Viola Reichenbachiana* Jord. and *Viola rupestris* Schmidt. A closely allied group of violets, represented in Britain by *Viola canina* L., *Viola lactea* Sm. and *Viola stagnina* Kit., differs in that a basal leaf rosette is not produced, and that the terminal bud of the main axis, as well as those of the numerous shoots produced laterally, itself lengthens into a flowering stem.

The following are some of the more important specific characters of *Viola Riviniana*:

Leaves cordate, only slightly longer than broad.

Upper stipules lanceolate often almost entire, not fimbriate.

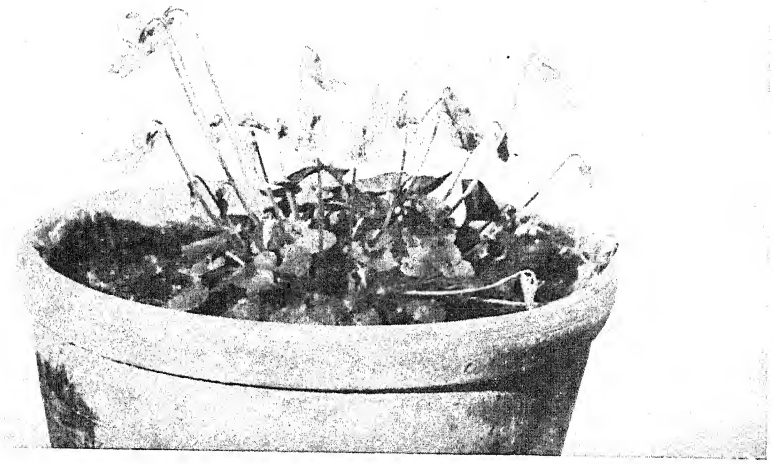
Calycine appendages conspicuous, about one-third to one-quarter as long as the sepals themselves.

Spurred petal comparatively broad (reaching 7 mm.) and richly veined.

Spur white or light purple, often furrowed at the tip and beneath, rather short and thick (e.g. 4·5 mm. long by 3 mm. deep).

The most reliable data as to geographical distribution are probably to be obtained from the monograph of Becker (1924), who studied *V. Riviniana* and its allied Asiatic species in great detail. He states that *V. Riviniana* is to be found over the whole of Europe. It extends to Portugal and Madeira in the south-west, and to Macedonia in the south-east. It reaches latitude 69° 42′ N. at Tromsö in Norway, and extends to about longitude 30° E. in Central Russia.

In the south-east of Europe, Becker recognizes a form, subspecies *neglecta* W. Becker, which first becomes distinct in the Balkans, and extends eastwards to Persia.



A. Potted plant of *V. Riviniana*, subspecies *minor*. Collected near High Cup Nick, Westmorland, May 1937. Photographed May 1938; slightly more than half natural size.

B. Potted plant of *V. Riviniana*, subspecies *nemorosa*. Collected at Eversden Wood, Cambs, in October 1936. Photographed May 1938; slightly more than half natural size.

SUBSPECIES *NEMOROSA* AND SUBSPECIES *MINOR* OF *VIOLA RIVINIANA*

We propose to use the term subspecies for the two types of *V. Riviniana* described below; we shall refer in the concluding remarks to the question of nomenclature.

The salient differences between characteristic specimens of subspecies *nemorosa* and subspecies *minor* are shown in the photographs (Pl. 2), which illustrate the plants as they appeared in the greenhouse in May 1938. Subspecies *nemorosa* is probably the more familiar; its leaves are comparatively large, its flowering branches long, and its flowers large and robust. The general habit is loose. In its spring state, subspecies *minor* is a compact plant, with much smaller leaves and shorter flowering branches than subspecies *nemorosa*, and with smaller and rather more delicate flowers. In detail, the flowers of subspecies *minor* differ mainly in having narrower and shorter petals, and narrower and shorter sepals; and the lower spurred petal rarely exceeds the lateral petals, and is less richly veined than in subspecies *nemorosa*. It must be remembered in looking at the photographs that both plants are much more vigorous and are flowering much more freely than is commonly the case in the field.

The same differences between the two subspecies are maintained throughout the growing season, although the summer leaves in subspecies *minor* are somewhat larger than the spring leaves, and the flowering branches longer. Subspecies *nemorosa* consistently has larger fruits and heavier seeds than subspecies *minor* and this is the case whether the fruits result from self- or cross-pollination.

Some figures indicating the order of difference in size between the two forms are given in Table 1.

Table 1

	Subspecies *minor*	Subspecies *nemorosa*
Dimensions of leaf on branch bearing open flowers (from two typical plants)	1·5 cm. long by 1·7 cm. broad	3·2 cm. long by 2·9 cm. broad
Dimensions of leaf on branch bearing cleistogamous flowers (from same two plants)	2·1 cm. long by 2·0 cm. broad	3·5 cm. long by 3·0 cm. broad
Length of branch bearing cleistogamous flowers	8 cm.	21 cm.
Distance from tip of spur to tip of lower petal	14–16 mm. (4 plants)	14–21·5 mm. (10 plants)
Length of lower sepal and its appendage	7·5–9 mm. (4 plants)	7·5–12 mm. (10 plants)
Breadth of upper petal	3·5–4·5 mm. (4 plants)	4–7 mm. (8 plants)
Length of capsule (from cleistogamous flowers)	6–8·5 mm. (6 plants)	9–13 mm. (10 plants)
Mean weight of a single seed	1·0–1·3 mg. (7 plants)	1·4–2·05 mg. (17 plants)

All the experiments that have so far been made indicate that subspecies *minor* and subspecies *nemorosa* breed true for their distinguishing characters. Many specimens of both subspecies have been under cultivation in the greenhouse for

periods varying from 18 months to 3 years, and during this time the characteristic differences have been maintained. Further, when plants of either form are selfed, the F_1 generation which is produced consists of plants almost indistinguishable from the parents. In the case of subspecies *nemorosa*, F_2 generations have also been obtained, and in these, too, the plants have bred true.

The results from subspecies *minor* are not yet complete; but Table 2 gives some idea of the type and extent of the differences between the offspring of *nemorosa* and *minor* subspecies. So far, the number of *minor* plants that has been tested in this way is five (from three localities) and of *nemorosa* plants, eight in the F_1 generation, and five F_2s; these *nemorosa* plants came from five different localities. More detailed data for different families of subspecies *nemorosa* will be given in a later section.

It has not been thought worth while to apply statistical tests to the data, as they are not yet extensive enough; nor has any detailed genetical work yet been done on individual characters. The data presented are intended to demonstrate the broad lines on which typical, selected specimens of subspecies *minor* and subspecies *nemorosa* differ from one another; and how the differences are maintained in their offspring. Population studies have been begun only on a small scale, and are at a preliminary stage.

Table 2

Young plants, grown from seed which germinated in May 1939; measurements made, 2 November 1939, of the maximum breadth, in cm., of the largest leaf on each plant.

Subspecies *nemorosa*: plants the product of selfing plants, themselves products of selfing a wild plant of *V. Riviniana* subspecies *nemorosa*, i.e. F_2 generation of subspecies *nemorosa*.

Subspecies *minor*: plants the product of selfing a wild plant of subspecies *minor*, i.e. F_1 generation of subspecies *minor*.

Leaf breadths, in cm., subspecies *minor*, F_1	Leaf breadths, in cm., subspecies *nemorosa*, F_2
1·65	2·9
2·1, 2·1, 2·1	2·95
2·2	3·15
2·25, 2·25, 2·25	3·2, 3·2, 3·2
2·3, 2·3	3·6, 3·6
2·35, 2·35, 2·35	3·7
2·4	3·8, 3·8
2·5	3·9
2·75	4·2, 4·2
Max. leaf breadth of parent, same date, 1·65	Max. leaf breadth of parent, same date, 2·6

The low values for the leaf breadths of the two parents are perhaps accounted for by the fact that the parents were in a cool greenhouse, while the seedlings and young plants had been grown in a warm greenhouse.

Although no measurements of the F_1 of this particular subspecies *nemorosa* plant were made, it was uniformly of a *nemorosa* type, like its parent and its offspring.

An examination of British herbaria has shown that the material of *V. Riviniana*

can be broadly classified, along the lines indicated, into subspecies *minor* and subspecies *nemorosa*.

Altogether, some thirty-six herbarium specimens of subspecies *minor* have been seen by the writer, from the counties of Devon, Cornwall, Dorset, Somerset, Sussex, Kent, Hertfordshire, Essex, Gloucester, Lake Lancashire, Westmorland, Cumberland, Yorkshire, Glamorgan; Co. Tyrone (Ireland); Selkirk, Forfar, Stirling, Shetlands and St Kilda. In addition, living specimens are in cultivation from Yorkshire, Glamorgan and Pembrokeshire; and at least six local floras refer to a plant which is undoubtedly subspecies *minor*. These floras are of Hertfordshire, Surrey, Glamorgan, Berkshire, Devon and Bristol. Subspecies *nemorosa* probably occurs in every county in Great Britain and Ireland.

Habitats of the subspecies of V. Riviniana. The plants of subspecies *nemorosa* and *minor* that have been described and figured come from two very distinct types of habitat.

Subspecies *nemorosa* comes from ash-oak woodland, with a shrub layer of coppiced hazel, growing on calcareous boulder-clay at 300 ft. in south-western Cambridgeshire. The following is a list of flowering plants in this habitat:

Quercus robur occ., dom.
Fraxinus excelsior coppiced, freq.
Corylus avellana abund.
Acer campestre loc. freq.
Crataegus monogyna occ.
Cornus sanguinea loc. freq.

Ficaria verna very abund.
Anemone nemorosa very abund.
Arum maculatum freq.
Poa trivialis freq.
Glechoma hederacea freq.
Ajuga reptans loc. freq.
Scilla non-scripta loc. abund.
Rumex viridis loc. freq.
Filipendula Ulmaria loc. freq.
Primula elatior loc. abund.
Viola Reichenbachiana freq.
Viola Riviniana freq.
Potentilla sterilis freq.
Fragaria vesca loc. abund.

The *p*H of the top 2 in. of soil in such a community varies from 6·5 to 7·0; and in winter, water is frequently within a foot or less of the surface.

Subspecies *minor* (though not the plant figured) comes from open grassland, grazed by sheep, on sugar limestone, at about 1700 ft. above sea level, in North-west Yorkshire. The turf is only an inch or two in height. The following is a list of flowering plants in this habitat:

Kobresia caricina
Sesleria caerulea
Festuca ovina
Helianthimum chamaecistus
Hieracium pilosella
Plantago maritima
Campanula rotundifolia
Linum catharticum
Potentilla erecta
Viola Riviniana

The *p*H of the surface soil, top 1½ in., is 7·6.

Neither of these lists is complete. They are included here to show the extreme difference between the types of community in which the subspecies of *V. Riviniana* may grow; they have been deliberately selected to emphasize this difference.

It is probable that this correlation between type of plant and type of habitat is general for the British Isles; for example Trow (1911) in *The Flora of Glamorgan*, notes that 'the very dwarf forms, growing in exposed situations, have very small leaves, stunted growth, etc.' We shall return to this point.

VARIATION IN THE SUBSPECIES OF *VIOLA RIVINIANA*, ESPECIALLY SUBSPECIES *NEMOROSA*

We shall give in this section a short account of some of the plants of subspecies *nemorosa* and *minor* that we have studied in the greenhouse. We shall deal first with typical subspecies *nemorosa*, such as we described in the last section; forms intermediate in some ways between *nemorosa* and *minor* are known, but they will be discussed later.

The following list indicates the nature and extent of variation in the plants of subspecies *nemorosa* that we have studied:

Habit. Flowering branches short (max. 4–5 in.) *or* long (max. 9–10 in.).
Flowering branches decumbent *or* ascending.

Vegetative reproduction. Plants constantly producing adventitious shoots from buds on the roots *or* never producing such shoots.

Behaviour during winter. Plants retaining leaf rosette and main bud over winter *or* death of rosette and bud, and new growth in spring by lateral shoots from the stock.

Leaves. Leaves wavy or crinkled in cross-section *or* leaves normal.

Hairs. Flower stalks covered with dense short hairs *or* flower stalks quite glabrous.

Sepals. Sepals (including calycine appendage) short (max. 7–8 mm.) *or* long (max. 11–12 mm.).

Petals. Upper petals narrow (4–5 mm.) *or* broad (7–8 mm.).
Lower petal obovate *or* obcordate *or* lanceolate.

Spur. Pure white *or* greenish white *or* light purple.
Straight *or* curved.

Style. Tip of style near stigma almost glabrous *or* hairy.

Seed. Seeds small and light (mean wt. = 1·4 mg.) *or* large and heavy (mean wt. = 2·1 mg.).

Anthocyanin in leaves. Leaves a dark coppery green in colour *or* leaves bright green.

Stipules. Broad *or* narrow.

The plants of *V. Riviniana* subspecies *nemorosa* on which this list is based have been drawn from a diversity of habitats and localities in the British Isles; but examples of nearly all the types of variation that are listed can be found in plants from the boulder-clay woodland of the Cambridge district. It appears therefore that both local populations and the population of the country as a whole show the same types of variation.

That the characters in the list are governed by genetical factors is shown by the fact that they are, almost without exception, heritable. (The more detailed results of a breeding experiment will be given below.) It is further found that the characters are combined together in different plants in all kinds of different ways, so that it is difficult to correlate any pair of characters. Certain combinations appear to occur more frequently than others, e.g. plants with light purple spur, large flowers, very long calycine appendages, ascending flowering branches, no adventitious shoots on the roots, and light seeds; also all the plants that we have

had in cultivation that have had hairy flower stalks have also had heavy seeds. But each fresh gathering gives plants different from any seen before. Many of the subtler characters have not, of course, been listed, especially those concerned with size of the leaves, flowers and branches, and the exact angle taken up by the flowering branches. The size characters are not only variable, but apparently are especially affected by environmental changes; it is therefore not easy to analyse them without very careful culture and measurement. Studies on single populations are only, as yet, at a preliminary stage; it is, however, possible to present a few data on plants of *V. Riviniana* collected from widely scattered localities in a single wood, viz. Buff Wood, East Hatley, Cambridgeshire.

Of the 16 plants originally collected in the summer of 1939, and since grown in the greenhouse, 4 died during autumn and winter. Of the 12 survivors, 9 are *V. Riviniana*, and 3 are the hybrid *V. Riviniana* Rchb. × *V. Reichenbachiana* Jord. This hybrid, which we shall refer to later, is common in Cambridgeshire woods; we have good evidence (which it is hoped to publish shortly) that the characters of *V. Riviniana* to which we shall refer here are dominant characters which always appear in the F_1 hybrid, so that we may include the data for the hybrid in those for the pure species.

The only two characters on which data are so far available are: retention *or* loss of main bud and leaf rosette during winter, and presence *or* absence of adventitious shoots on the roots. Of the 12 plants, 7 lost their main bud and rosette, while 5 retained them; and 4 have produced adventitious shoots from the roots, while 8 have not.

We shall now examine two plants in some detail, in order to give a more precise picture of the differences within subspecies *nemorosa*, and of the way in which these differences are inherited.

The two plants we have selected come from Little Widgham Wood, Cambridgeshire, and from Howe Wood, Essex, and were called respectively Little Widgham B, and Howe C. Both were collected in 1936, and have been in cultivation in the greenhouse ever since. Seeds from self-pollinated cleistogamous flowers were sown in 1937; the F_1 plants were grown in 1938, and came to maturity in 1939.

Many of the differences between the plants, and the extent to which they are inherited, will be clear from Table 3.

Seed weight and percentage germination.

	Seed weight mg. mean of 50	Percentage germination 20–30 seeds
Little Widgham B	1·5	55
Little Widgham B F_1	1·5	80
Howe C	1·6	60
Howe C F_1	1·6	67

It is clear that in these characters both plants differ little and breed true.

Production of adventitious shoots on roots. Of the parents, Howe C has never,

in the period 1937–40, produced adventitious shoots on the roots, while in Little Widgham B, adventitious shoots have been observed in 1938, 1939 and 1940.

In the F_1 offspring of Howe C, of which eleven survived, adventitious shoots on the roots have only been observed once in one of the plants, and this observation, made by an assistant, requires confirmation.

In the F_1 offspring of Little Widgham B, of which eleven survived, six in the summer of 1939 produced adventitious shoots on the roots, and five did not.

It is fairly clear from these results (and this is confirmed by many other data) that the ability to produce adventitious shoots on the roots is a heritable character.

Table 3

	Little Widgham B 7. v. 37	Little Widgham B F_1 16. v. 39	Howe C 22. v. 37	Howe C F_1 22. v. 39
Habit	Shoots decumbent and slightly ascending	Flowering branches beginning to ascend	Stems decumbent or ascending	Flowering branches ascending slightly
Petals	Upper and lateral petals dark lilac, lateral darker at base	Petals dark lilac, not darker at the base	Upper and lateral petals dark lilac, darker at the base. Upper with two veins showing faintly at the base, lateral with two or three dark distinct veins at the white base	Petals rather dark lilac, not or barely darker at the base. Upper and lateral petals with dark veins extending to about half-way and then fading out
Leaves	Normal, not wavy	Normal, not wavy	Somewhat wavy in cross-section	Markedly wavy in cross-section
Spur	White, faintly tinged with green, greenish furrow below, rather distinctly bilobed at tip	Practically pure white, narrowing at the tip and there swelling slightly and becoming slightly furrowed	Reddish-purple at base, becoming very light purple at the tip; grooved below, and unequally lobed at tip	Light grey-purple, tapering, furrowed at tip

These descriptions are extracted from laboratory note-books; the descriptions of the F_1 plants were made 2 years after those of the parents and quite independently of them. The particular F_1 plants described were selected at random.

Behaviour during winter. The plants have remained in the greenhouse, which is unheated, during the winter. There is no doubt that variation can occur in a single plant as regards winter behaviour; for example, in the winter of 1938–39, Howe C retained its terminal bud, whereas in the winter of 1939–40, this bud was lost, and a new bud developed laterally from the stock at ground level. It is, however, probable that this behaviour in 1939–40 was abnormal and connected with accidental damage to the plant, which led to its weakening in the autumn of 1939. Of the seven plants of the F_1 of Howe C that survived the winter of 1939–40, six retained their terminal buds and also some of their rosette leaves, and in one only was the terminal bud killed; this plant is regenerating by means of a lateral bud from the stock.

Unfortunately, we have no data for the winter behaviour of the F_1 offspring of Little Widgham B, but we may mention those of Little Widgham A, a plant which is very similar indeed to Little Widgham B. In Little Widgham A itself, in two successive winters, the terminal bud has been killed, the rosette leaves have disappeared, and the plant has regenerated by new lateral buds from the stock. This has been precisely the behaviour of all of the plant's five surviving F_1 offspring.

There is thus good reason to believe that the way in which *V. Riviniana* reacts to winter conditions is governed by heritable factors; and this view is reinforced by evidence from other breeding experiments. It is interesting to note that in Little Widgham A and its offspring, which, like Little Widgham B, habitually produce adventitious shoots from the roots, regeneration in spring is by means of new shoots from the stock, and *not* by new shoots from the roots.

Flower characters. The difference of *spur colour* in the plants will be clear from Table 3. We may add that all the surviving F_1 offspring of Howe C had a light purple spur, and that all those of Little Widgham B had a white spur with the exception of one in which the spur was very slightly tinged with purple.

The way in which petal characters such as shape and venation are inherited has also been indicated in Table 3. We present here, in addition, further data on sepal and spur characters. These data are set out in Table 4.

Table 4

Name of plant	Length in mm. of lower sepal (x)	Length in mm. of appendage of lower sepal (y)	Ratio x/y	Shape of spur
Howe C, F_1, No. 65 J	7·75	3·0	2·6	Curved
Howe C, F_1, No. 65 K	7·5	3·75	2·0	Curved, tapering
Howe C, F_1, No. 65 B	6·75	3·5	1·9	Markedly curved
Howe C, F_1, No. 65 A	6·75	3·25	2·1	Curved, tapering rather abruptly
Howe C, F_1, No. 65 G	6·5	3·0	2·3	Curved
Howe C, F_1, No. 65 H	6·5	2·75	2·4	Curved, tapering
Howe C, F_1, No. 65 F	6·25	3·0	2·1	Curved, tapering rather abruptly
Howe C, F_1, No. 65 D	6·0	2·0	3·0	Curved
Little Widgham B, F_1, No. 62 A	7·75	2·5	3·1	Straight
Little Widgham B, F_1, No. 62 G	7·5	2·5	3·0	Straight, but in some flowers tapering
Little Widgham B, F_1, No. 62 J	7·5	1·5	5·0	Straight
Little Widgham B, F_1, No. 62 D	7·25	2·25	3·2	Straight
Little Widgham B, F_1, No. 62 K	7·25	1·5	4·8	Straight
Little Widgham B, F_1, No. 62 B	7·0	2·0	3·5	Straight
Little Widgham B, F_1, No. 62 L	6·75	1·75	3·9	Straight
Little Widgham B, F_1, No. 62 C	6·5	2·0	3·3	Straight
Howe C, from measurements made 22. v. 37	6·0	2·5	2·4	Curved, tapering
Little Widgham B, from measurements made 7. v. 37	8·25	2·5	3·3	Straight

Flowers collected from the F_1 plants in June 1939, and preserved in alcohol. Between two and ten flowers collected from each plant. Measurements made January 1940. For the measurements, a single flower was taken from the batch for that plant; about the largest flower was selected, but withering flowers were not used.

The difference in shape of spur, which was characteristic of the parents, is clearly shown in all the offspring.

It is clear, too, that the sepal characters of the parents are also inherited; the ratio, length of sepal : length of appendage, is clearly significantly greater in the offspring of Little Widgham B than in that of Howe C.

Variability of subspecies minor. It is highly probable that subspecies *minor*, as we have defined it, is just as variable a plant as subspecies *nemorosa*, although we have not yet observed, in cultivation, nearly so many types, or types from so many localities, as of subspecies *nemorosa*. However, variability has been observed in the following characters:

Vegetative reproduction	Colour of spur
Length of sepals	Colour of leaves
Breadth of stipules	

Also, breeding experiments with subspecies *minor* are not yet so far advanced as those with subspecies *nemorosa*; it seems probable, however, that individuals of subspecies *minor* will be just as true-breeding as those of subspecies *nemorosa*.

Intermediates between subspecies nemorosa *and* minor. So far, we have drawn a sharp distinction between subspecies *nemorosa* and *minor*; but in reality the two are connected by a series of intermediates. These plants of *forma intermedia*, as we may call them temporarily for convenience, are plants which, so far as our experiments go, appear to breed true for their intermediate *size* characters, at any rate. For example, we quoted in Table 2 figures for F_1 and F_2 families of *minor* and *nemorosa* plants. The leaf breadths in the *minor* family ranged from 1·65 to 2·75 cm. (20 measurements), mean 2·25 cm., and in the *nemorosa* family from 2·9 to 4·2 cm. (14 measurements), mean 3·55 cm. For an F_1 family of *forma intermedia*, we found a range of 2·2–3·1 cm. (20 measurements), mean 2·7 cm. These plants of *forma intermedia* were intermediate between *nemorosa* and *minor* in other characters; e.g. they resembled subspecies *nemorosa* in their long flowering branches, but subspecies *minor* in their narrow petals.

It is of interest that plants of *forma intermedia* frequently come from types of habitat which are intermediate between those typical of subspecies *nemorosa* and *minor*. For comparison with the data given on p. 193, some field notes are quoted here on the habitat of the parent plant of the F_1 family of *forma intermedia* that has just been mentioned.

This plant of *forma intermedia* came from grassland near the coast, at St Ann's Head, Pembrokeshire. The grass was thick and about a foot high; the soil was brown, sandy and friable. The violet grew rather deep down in the grass. The following is a list of plants in the habitat:

Agrostis tenuis dominant	*Potentilla erecta* freq.
Holcus lanatus abundant	*Centaurea nigra* freq.
Carex Goodenowii loc. freq.	*Festuca rubra* freq.
Vicia Cracca freq.	*Plantago lanceolata* freq.
Viola Riviniana freq.	*Lotus carniculatus* occ.
Calluna vulgaris frequent small bushes	

We have thus described the three forms, *nemorosa*, *intermedia* and *minor* as coming from, respectively, a very sheltered woodland habitat, a habitat in a rather exposed place on the coast, but in long grass, and a habitat in short turf on an exposed hillside.

We are, of course, lumping together under the term 'exposure' many physical factors such as temperature, humidity and light intensity; and we do not intend to suggest that even a good correlation is to be found, in all cases, between type of plant and type of habitat. Indeed, it would be a very great labour to demonstrate this correlation on a large scale, for not only would it involve the gathering and cultivation of many plants from a diversity of localities; it would also involve an ecological analysis of the habitats in all these localities. As every ecologist knows, it is a task of great difficulty to get a satisfactory series of data which will describe the physical conditions of a habitat.

As matters are at present, then, we have to be content with a very rough ecological analysis of the type that we have already given; and with a limited amount of analysed plant material. Herbarium specimens of *V. Riviniana*, of which a great many are available, are of some use; but the extraordinary plasticity of the plant, and the large seasonal changes which it shows, make cultivation in the greenhouse the only conclusive method of study.

We have been able to make artificial hybrids between subspecies *nemorosa* and subspecies *minor* in the laboratory; these hybrids, however, have not yet come to maturity, and a full study of them has yet to be made. The hybrids appear to be quite fertile; their offspring, i.e. the F_2 of the cross, is at present only in the seedling stage.

Cytology

Several workers have examined the chromosomes of *V. Riviniana*. Clausen (1927) investigated meiosis in plants from beechwood in Seeland, Denmark, and found $n=20$. West (1930) examined wild plants, from a locality which was not given, both of the type and of var. *nemorosa* N. W. et M.; she observed twenty bivalents at meiosis and twenty chromosomes in pollen-grain division. Gershoy (1937) examined plants which had been sent to him by Clausen from the botanical garden of the University of Copenhagen, and found $2n=40$ in root-tips.

Although there was this general agreement as to the chromosome number, viz. $2n=40$, it was thought advisable to make counts of our own material. Two plants were selected, one of subspecies *minor*, and one of subspecies *nemorosa*. Root-tips were fixed in the modification of Navashin's solution known as Craf (La Cour, 1937); they were cut at 15μ, and stained with gentian violet and iodine according to the schedule given by La Cour.

We found, for the plant of subspecies *minor*, $2n=40$, and for the plant of subspecies *nemorosa*, $2n=46$. This result was, of course, unexpected; it has been checked, indirectly, by counting root-tip chromosomes in two hybrids, made artificially in the greenhouse, of *V. Riviniana* × *V. Reichenbachiana* Jord. *V. Reichenbachiana* is a species which has been studied by us for some time, and it is hoped

to give a detailed account of its hybrid with *V. Riviniana* in another place. It must suffice here to say that the two species hybridize together very readily, and the hybrids are sterile.

The chromosome number of *V. Reichenbachiana* is known both from the accounts of previous workers (Clausen, 1927, etc.) and from our own counts to be $2n=20$; hence its hybrid with *V. Riviniana* may be expected to have $2n=30$.

One of the hybrids whose chromosome number we counted had for one of its parents (female parent) the plant of *V. Riviniana* with a number of $2n=46$; the number found for this hybrid was $2n=34$. The other hybrid had as its male parent another plant of *V. Riviniana*, subspecies *nemorosa*; this hybrid had a number of $2n=30$, and we can therefore deduce that the chromosome numbers of its parents were $2n=40$ (*V. Riviniana*), and $2n=20$ (*V. Reichenbachiana*).

The following table summarizes these results:

Table 5. *Chromosome numbers in* V. Riviniana *Rchb.*

Author	Locality	Number *n*	2*n*
Clausen, 1927	Seeland, Denmark	20	—
Clausen, 1927	Seeland—intermediate between *V. Riviniana* and *V. Reichenbachiana*	About 20, some univalents	—
West, 1930	England	20	—
Gershoy, 1937	Botanical Garden, Copenhagen	—	40
Valentine, 1940	Widdy Bank Fell, Teesdale, Yorks—subspecies *minor*	—	40
Valentine, 1940	Little Widgham Wood, Cambs, subspecies *nemorosa*	—	46
Valentine, 1940	Little Widgham Wood, Cambs, subspecies *nemorosa* (deduced from hybrid with *V. Reichenbachiana*)	—	48
Valentine, 1940	Howe Wood, Essex (deduced from hybrid with *V. Reichenbachiana*)	—	40

We have, as yet, made only preliminary investigations of meiosis in *V. Riviniana*; and until this has been completed, it is difficult to draw any final conclusions from Table 5. We have included in the table observations of Clausen's on some plants which he considered to be intermediate in appearance between *V. Riviniana* and *V. Reichenbachiana*; he found irregularities in the meiosis of these plants. They had about twenty bivalents, but sometimes less, and a variable number (2–3) of univalents. Clausen regards these plants, on the basis of a theory which we need not discuss here, as products of the hybrid *V. Riviniana* × *V. Reichenbachiana*. It is possible that the irregularities in chromosome number in *V. Riviniana* which we have described correspond with the irregularities observed by Clausen in his Danish plants. It must be emphasized, however, that the plant with the number $2n=46$ is not only morphologically *V. Riviniana*, but is perfectly fertile both when selfed and crossed; in contrast, both the F_1 *V. Riviniana* × *V. Reichenbachiana*, and its F_2 offspring, are highly infertile.

It will be noted that the chromosome number of the plant from Little Widgham Wood is found, by direct counting, to be $2n=46$, and, by deduction from its hybrid, $2n=48$. The explanation of this fact must wait upon investigation of

meiosis; we have, as yet, no idea of the proportion of plants of *V. Riviniana* with the different chromosome numbers.

It is worth noting that *V. canina* L. appears to vary cytologically in a way that is analogous to *V. Riviniana*. Clausen (1931) reported a number of about $2n=47$ for Danish material of *V. canina*, whereas Bruun (1932) found $2n=40$ for *V. canina* from Upsala. In this case, too, the true reason for the variability in chromosome number has not been made clear; Clausen (1931) suggests that its origin lies in hybridization between *V. Riviniana* and *V. canina*. These results will be further discussed in the Concluding Remarks.

Taxonomy

It must be remembered, in this section, that the names subspecies *nemorosa* and subspecies *minor* are used for the first time in this paper; they are based on *forma nemorosa* N. W. et M. and *forma minor* Murbeck, which will be described below, and which cover a much narrower range.

As yet, we have had little opportunity of investigating *V. Riviniana* either alive, or in the dried state, outside Great Britain; and the foreign herbarium material available is too scanty to permit of definite conclusions. Beyond saying that, as well as subspecies *nemorosa*, subspecies *minor* certainly occurs on the Continent under a bewildering variety of names not quoted here, we shall confine our discussion to the taxonomy of British material. We may perhaps add that Becker (1924), monographer of the genus *Viola*, in his final account of the section to which *V. Riviniana* belongs, apparently neglects the *minor* forms altogether, and lists only var. *typica* N. W. et M. (British), var. *nemorosa* N. W. et M. (British), and var. *trichocarpa* W. Beck., a very hairy form which is unknown in Britain.

We shall deal first with forms in which there is no difficulty in identification. *Forma rosea* N. W. et M., with reddish flowers and *forma luxurians* Beck. with pure white flowers are recorded by Hall (1939) in the *Flora of Devon*; we have not had either of these forms in cultivation, but we may reasonably expect them to be true breeding for their colour characters. *Forma villosa* N. W. et M., with hairy peduncles, has already been mentioned by us under subspecies *nemorosa*; and a small F_1 generation which we raised from a plant of *forma villosa* had hairy peduncles like the parent.

Gregory (1912), in her monograph on British violets, describes a plant under the name var. *pseudomirabilis*, and identifies it both with the *V. pseudomirabilis* of Coste and with *V. silvatica* Fr. var. *pseudomirabilis* A. et G. With the former it is certainly not identical, with the latter possibly so. We have seen Gregory's specimens; their resemblance to true *V. mirabilis* L. is superficial, and we have no hesitation in diagnosing them as a variant of *V. Riviniana* subspecies *nemorosa*, of similar status to the numerous variants such as we have already described on p. 194. The distinguishing features of Gregory's plants are the rather broad leaves, which are very rounded at the apex and not produced into the usual blunt point.

Three other named types remain to be examined, viz. *forma nemorosa* N. W. et M., var. *diversa* Greg., and *forma minor* Murbeck. We shall deal with them in that order.

Forma nemorosa N. W. et M. The original description (1886) runs as follows: 'Appendicibus sepalorum plerumque brevioribus, petalis paullo angustioribus, violaceis, prope basim macula obscuriore instructis, calcari violaceo.' The plants described, and distributed, came from Swedish woodland; and they belong with doubt to subspecies *nemorosa* nobis.

Neumann, Wahlstedt and Murbeck's original specimens have been examined in the Herbarium at Kew, as well as their specimens of *forma typica* N. W. et M., which come from a different locality. The specimens of the two *formae* are probably representative of two populations of *V. Riviniana*, which the authors observed were constantly different; but we doubt very much whether the particular combination of characters to which they give the name *nemorosa* is any more worthy of a name than a great many other, and different, combinations which certainly occur.

Gregory (1912) interprets *forma nemorosa* as meaning plants with shorter calycine appendages than those of typical *V. Riviniana* (half-way between those of *V. Reichenbachiana* and *V. Riviniana*), and which flower later than the type. Time of flowering is undoubtedly a character of *V. Riviniana* which varies greatly, though we have not yet been able to gather sufficient data to demonstrate that it is heritable.

P. M. Hall (1939), in the *Flora of Devon*, refers to *forma nemorosa* N. W. et M. as 'a critical form, showing transitional characters towards *Viola Reichenbachiana* in its foliage, small sepaline appendages and coloured spurs'. In another place, Hall (1928) describes both the type and *forma nemorosa* as growing in the same wood, and remaining perfectly distinct; he mentions in addition, as a character of *forma nemorosa*, that 'the leaves have longer and more tapering apices (than the type; D.H.V.), resembling those of *Viola Reichenbachiana* in outline'. We feel that Hall's opinion, as an expert on the taxonomy of British Violas, must carry great weight; and we might even not dissent from his opinion (Hall, 1928) that *forma nemorosa* is worthy of varietal rank. But if this be accepted, then we should feel compelled to create a great many more varieties based, for example, on the characters which we have listed on p. 194.

Forma minor Murbeck. Hall (1939) defines this as 'a dwarf form of exposed places, upland pastures, sea-cliffs, etc., having small dark foliage, and few, large, brightly coloured flowers, with yellow spurs'. Gregory (1912) says also that 'the plant remains dwarf after the flowering season is over'. This plant clearly comes under subspecies *minor*; we have not had specimens in cultivation with yellow spurs, nor can the subspecies *minor* we have described be said to have 'large' flowers; nevertheless we have seen numerous herbarium specimens which have been labelled *forma minor* Murbeck which have not had 'large' flowers, and which also clearly come under subspecies *minor* nobis.

Var. *diversa* Greg. The original description (Gregory, 1912) runs: 'Primo vere pusilla, erecta, congesta, floribunda; foliis parvis, floribus parvis, vario colore tinctis, petalis procurrentibus; mox diffusa foliis floribusque crescentibus, sed petalis aliquanto minus patulis quam in Viola Riviniana typica.' Hall (1939) says that var. *diversa* Greg. is 'the form which occurs (in Devonshire; D.H.V.) abundantly

on heaths, moors, etc. It is characterized by its dwarf floriferous habit, with a wide range of variation in the colour of the flowers and spurs.'

As for *forma minor* Murbeck, it is clear from these descriptions that var. *diversa* Greg. comes within the range of the type that we have called subspecies *minor*; and it is also clear that the habitat of the plant is used by Hall as a character in the definition of both. This, indeed, corresponds closely with what we have described as the 'exposed' habitats of our subspecies *minor*, as contrasted with the 'sheltered' habitats of subspecies *nemorosa*. As regards size of flower, it is clear that var. *diversa* Greg. corresponds better with our subspecies *minor* than does *forma minor* Murbeck. When, however, we come to examine accredited specimens of var. *diversa* Greg. in British herbaria, we find that it frequently corresponds with our *forma intermedia* rather than with our subspecies *minor*.

Hall (1928) admits that subspecies *minor* Murbeck and var. *diversa* Greg. 'in certain situations approximate very closely to one another'; and it is fairly clear that no sharp line can be drawn between them. We have collected in the field plants that correspond closely with *forma minor* Murbeck, and which, on cultivation in the greenhouse, correspond more closely with var. *diversa* Greg.

As regards the variation in colour of the flowers of var. *diversa*, we have observed colour variations in different plants of our subspecies *minor* in the greenhouse, and similar variations in our subspecies *nemorosa* too. As regards size of flower, we cannot help thinking that use of the terms 'large' and 'small' has been responsible for a good deal of confusion. There is a specimen in the herbarium of E. S. Marshall, at Cambridge, which has been identified as *V. Riviniana forma minor* Murbeck by H. J. Riddesdell, E. S. Gregory, and Marshall himself. Marshall's note to the specimen runs: 'the rather *large* flowers are against its being var. *diversa* Greg.' Yet the flowers are of the size which we have called, in this paper, *small*, i.e. they fall within the range of our subspecies *minor* as it is defined on p. 191.

As regards the distinction, made by Gregory, that the size of plants of var. *diversa* increases considerably as the season advances, while *forma minor* Murbeck remains small, we can only say that, in cultivation, and, so far as we have observed them, in the wild as well, plants of *V. Riviniana* nearly always increase in size during the summer. The flowering branches elongate; and the summer leaves are nearly always bigger than the spring leaves. The percentage change in size is often, but not universally, greater in subspecies *minor* than subspecies *nemorosa*. Some typical figures for leaf breadth are given in Table 6. Our experiments, however, provide no evidence for the distinction, based on seasonal change, which is made by Gregory.

Table 6. *Measurement of breadth, in cm., of the six broadest leaves on two flowering branches taken at random.* (*Preserved material*)

	Plant of subspecies *minor*	Plant of subspecies *nemorosa*
May 1938	1·85, 1·9, 1·9, 1·9, 2·0, 2·1	2·4, 2·8, 2·8, 2·9, 3·0, 3·5
August 1938	2·15, 2·25, 2·3, 2·4, 2·45, 2·6	2·85, 3·0, 3·2, 3·25, 3·3, 3·45

There is evidence, from a large number of herbarium specimens, that Mrs Gregory identified many British violets as varieties or forms of *V. rupestris* Schmidt. For example, in Herb. Druce, five specimens collected from the Shetland Islands by G. C. Druce in 1924 are identified by Gregory as *V. rupestris* Schmidt var. *glabrescens* Neum.

All specimens referred to this species that we have seen, from the British Isles, belong without doubt to *V. Riviniana* subspecies *minor* nobis, with the exception of specimens of *V. rupestris* Schmidt var. *arenaria* D.C. from the classical locality of Widdy Bank Fell, Teesdale, where both *V. rupestris* and *V. Riviniana* subspecies *minor* occur together. Notes made by P. M. Hall on herbarium sheets leave no doubt that he also regards Gregory's identification as erroneous.

CONCLUDING REMARKS

Three main sets of factors appear to underlie the variation of *V. Riviniana* in Britain, ecological, genetical and cytological.

Ecological. It has been pointed out that subspecies *minor* and *nemorosa* are characteristic of two distinct types of habitat, roughly defined as 'exposed' and 'sheltered' respectively. This brings them into Turesson's category of ecotype (Turesson, 1922), of which it is sufficient to say that ecotypes are genetically distinct forms of species, characterized by constant habitat differences, just as are the subspecies of *V. Riviniana*; and that they probably arise from a stock which splits under the selective influence of the environment.

In many parts of East Anglia, e.g. parts of Cambridgeshire and West Suffolk, subspecies *minor* is absent and only subspecies *nemorosa* occurs, mainly in woodland; we must presume that in these parts, *V. Riviniana* is unable, under present conditions, to survive at all outside woodland, and that the evolution (or invasion) of subspecies *minor* cannot therefore occur. For example, there are large areas in the Breckland of West Suffolk which might be thought suitable for subspecies *minor*; but it does not occur, and in situations where it might be expected, a form of *V. canina* L. with a similar habit occurs instead.

As we go westwards, we find that *V. Riviniana* is no longer confined to woodland; and on the open heaths and cliffs of Wales, for example, we find an abundance of *V. Riviniana*, usually as subspecies *minor*. Subspecies *nemorosa* is still present in the woodland, and is now more obvious in the hedgebanks. Some factors in the environment have changed, climate probably the most important, in going from east to west, with the result that *V. Riviniana* is able to emerge from sheltered habitats, and in the more exposed habitats to be transformed into subspecies *minor*. It is possible that subspecies *minor* has arisen in this way several times over in different places; it is possible too that the transformation, at least in some cases, has been fairly recent, and that it has taken place during the removal of woodland from the countryside, a process that has been most rapid during the last few hundred years.

We have noticed (p. 198) the occurrence of a *forma intermedia*. If the facies of *V. Riviniana* is correlated with the type of environment in which it occurs, this is

perhaps to be expected, viz. a form of the plant intermediate in character between subspecies *nemorosa* and *minor*, and growing in a habitat neither fully exposed nor fully sheltered. As we have already pointed out, we run into difficulties here when we attempt to define the type of habitat. These observations fall very naturally into the concept of 'cline'. Clines have been recently defined by Huxley (1940) as 'character gradients within groups'.

One of the great advantages of the concept of the cline is that it allows correlation of a *range* of habitats with a *range* of varying forms. Thus, to apply it to *V. Riviniana*, at one end of the cline is subspecies *nemorosa*, in its sheltered habitat, and at the other, subspecies *minor*, in its exposed habitat; in between lie a series of forms (called in this paper *forma intermedia*) and many intermediate habitats, and these now range themselves along the cline. It is called here an ecocline, as it is based partly on ecological criteria. We must, of course, point out that this ecocline in *V. Riviniana*, though probable and supported by some evidence, cannot be regarded as fully demonstrated. Reference should be made to the work of Gregor (1939) for an account of clines in *Plantago lanceolata* L.

Cytological. The data we have presented on the cytology of *Viola Riviniana*, though scanty, indicate a type of variation perhaps quite different from that mentioned under the ecological heading; indeed the boundaries which the cytological data indicate are not only obscure, but may well have little relation to the ecotypes.

The origin of the extra chromosomes is a puzzle, to which the answer may lie in a previous extra-specific cross. It is perhaps significant that the single aberrant type so far discovered ($2n=48$) came from a wood in which *V. Reichenbachiana* Jord., and doubtless too its hybrid with *V. Riviniana*, also occurs. This hybrid has a diploid number of 30 chromosomes, and at reduction division there are formed 10 bivalents and 10 univalents; the former divide regularly, the latter appear to scatter at random. Thus gametes with $(10+x)$ chromosomes are produced, which are usually non-viable. However, viable gametes are sometimes formed, for occasionally the hybrid produces seed which will grow into a plant rather similar to its parent. It is a possibility that an unreduced gamete of this hybrid with 30 chromosomes, such as is known to occur sometimes among flowering plants, might be fertilized in a cleistogamous flower by a viable $(10+x)$ gamete. This would give a $(40+x)$ plant corresponding to the plant with 48 chromosomes that we have described; and this plant might have *V. Riviniana* characters heavily predominant over those of its other remote parent, *V. Reichenbachiana.*

There is as yet no evidence for this hypothesis, and it would not be justifiable to discuss it in more detail. It is, however, suggestive; if it were found to be correct, then we might expect to find aberrant cytological forms of *V. Riviniana* only where *V. Reichenbachiana* grows and there are opportunities for hybridization. It is worth noting too that it is only subspecies *nemorosa*, in this country, that ever comes into contact with *V. Reichenbachiana*, which is a woodland plant; subspecies *minor* comes into contact with quite a different species, viz. *V. canina* L. ($2n=40$), and hybridizes with it. It was among a population of *V. canina* in contact with

V. Riviniana that Clausen discovered his aberrant *V. canina*, with $2n=40+x$ chromosomes. Here again there is strong suggestion that hybridization has had something to do with the chromosomal aberration.

V. Riviniana may thus be capable of giving rise to two quite different sets of hybrids, which, in their turn, may settle down to some sort of stability, either as forms of *V. Riviniana* or of the other species with which the cross was made. Hybrids of *V. Riviniana* are recorded with at least seven species in Europe; so that there is a large mass of hybrid material here from which new combinations can develop.

Genetical. The state of affairs in *V. Riviniana* that we have described may be summarized in this way. *V. Riviniana* is a species, well marked off, in Great Britain at least, from its close allies, yet within itself, heterogeneous. Part of this heterogeneity we have already accounted for, by describing the two subspecies as ecotypes, which have evolved, by a process of natural selection, in two different types of environment. Cytological heterogeneity has been discussed, and as yet its connexion with morphological variation is obscure. There still remains a large stock of heterogeneity which is unexplained. In an earlier section (p. 194), the variable characters of *V. Riviniana* were described. It was stated that similar variations were noticeable in both subspecies, and that many plants had been found in which these characters were combined together in many different ways; and it was further shown that some plants bred fairly true for particular character combinations.

Dobzhansky (1937), in a discussion of variation in natural populations, draws attention to the work of Wright and others on the 'scattering of variability', i.e. the way in which a population attains complete genetic uniformity; this is specially marked in small populations, where, unless the mutation rate is exceptionally high, one allelomorph of every gene displaces, by chance, all the others until the whole population is homozygous for all its genes. We do not know yet whether such a process is going on in *V. Riviniana*, as we have made only preliminary population studies, but in Buff Wood there is, as we have pointed out (p. 195), still a considerable stock of variation and the *population* is by no means homozygous for all its genes. It is possible, however, that some individuals in the population are homozygous, and this may be due, partly to the restriction of population size (confined to a single wood), and partly to a different factor, similar in effect, viz. inbreeding. Close inbreeding naturally leads soon to homozygous individuals; in its effects, it is equivalent to a reduction in population size.

V. Riviniana is a species in which inbreeding is common. Early in the year, open flowers are produced, which are normally cross-pollinated, though if artificially selfed, they are fertile. Later in the year, cleistogamous flowers occur, which are self-pollinated, and produce fertile seed. Numerous observations in the field and the greenhouse lead to the view that open flowers rarely set seed unless they are 'disturbed' in some way, and this 'disturbance' will usually be by an insect visitor. We may therefore assume that some at least of the fruits from open flowers result from cross-pollination.

Table 7 shows, therefore, that seed is set, in a favourable year, from both cross-

and self-pollination; numerous greenhouse experiments have shown that both sorts of seed are viable and give high percentage germination.

Table 7. *Observations on plants of* V. Riviniana *at four localities in Buff Wood, Cambridgeshire*, 1939

	Number of plants	Number of open flowers on 12 May	Total number of fruits from open flowers observed on 12 May and 8 June	Total number of fruits from cleistogamous flowers observed on 8 June and 8 August
Locality A	4	23	3	8
Locality B	6	15	11	5
Locality F	6	20	10	32
Locality G	3	12	11	18
Totals	19	70	35	63

It is thus fairly certain that, in some situations at least, there will be much inbreeding in *V. Riviniana*; and it is therefore to be expected that individuals which appear to be practically homozygous for most of their characters will occur. On the other hand, it is fairly certain that cross-pollination does occur, and this will produce new gene combinations, and new types. The situation will, of course, also be affected by the size of the population, which varies greatly in different situations, e.g. a small population in a single fragment of woodland in East Anglia, and a large population on an extensive stretch of cliff in the West of England.

It is hardly surprising, then, that we find an enormous number of slightly differing types in different parts of the country and that these are not easy to classify; the complete analysis of a single population will itself be a complicated task.

Our discussion so far has been confined to *V. Riviniana* in Britain. We have as yet little to add with regard to its status in Europe. Two points may be mentioned.

First, both subspecies *nemorosa* and *minor* occur on the Continent of Europe. For example, the Central European plants mentioned by Hegi (1931) as *forma bryophila* W. Becker and *forma arenicola* Chabert probably belong to subspecies *minor*. Most of the European herbarium material we have seen belongs to subspecies *nemorosa*, but there are specimens of subspecies *minor* in Herb. Kew from Iceland and from Italy.

Secondly, it is possible that there is a topocline as well as an ecocline, i.e. that as the species goes eastwards and southwards, it changes gradually, until, in the Balkans, it becomes sufficiently distinct to be described as a separate subspecies, subspecies *neglecta* W. Becker. This again is only a hypothesis; and other geographical races may exist. We have seen some specimens from Portugal which differ from most British material in their broader and rounder leaves, and which may constitute a distinct group.

We turn finally to a discussion of the relation of our conclusions to the orthodox taxonomy and nomenclature of the species. It is clear, from the section on taxonomy, that these conclusions are not in bad accord with the existing taxonomy. It is equally clear that there are a good many named forms in Britain, and consultation of French and German floras shows that there are still more named forms. Many of these are probably given merely to small, local populations, or to types of plant that crop up sometimes in many populations. Interesting and worthy of study as these are, it is only confusing to give them names without examining them in detail, and determining their status.

We propose, for the time being, two names only for general use, viz. subspecies *nemorosa* and subspecies *minor*. Both have already been described in this paper, but they may be briefly redefined here as follows:

Subspecies *nemorosa* N. W. et M. emend. Valentine. Leaves large, typically about 3 cm. in length and breadth. Flowering branches long, typically more than 15 cm. Flowers long, often exceeding 20 mm. in length. Mature seeds heavy, typically 1½–2 mg. in weight. Living in sheltered habitats, typical of woodland.

Subspecies *minor* (Murbeck) Valentine. Leaves small, typically 1½–2 cm. in length and breadth. Flowering branches short, and typically about 10 cm. Flowers short, typically about 15 mm. in length. Mature seeds light, typically 1–1½ mg. in weight. Living in exposed habitats.

It is hoped to publish soon a short paper, clearing up details of nomenclature.

The number of terms for units below the rank of species is very great, and new criteria have led to new terms such as the ecotype (Turesson, 1922), and the convivium (Danser, 1929). These new terms clash with the older terms, such as subspecies, variety and form; and we are faced with a situation in which the same plant of *V. Riviniana* can be labelled ecotype *minor*, subspecies *minor*, variety *diversa*.

Perhaps the best way of solving the difficulties of this situation, and one that has the advantage of practical convenience, is to stick to the old terminology, but to widen and extend the meaning of these terms by redefining the units they represent in new ways, so as to include, for example, the ecological information represented in the ecotype of Turesson. It is for this reason that we have used the term subspecies in this paper. Huxley (1940) recommends that the term 'variety', which has been used in so many senses, be dropped. This is probably impossible; 'variety' is too well entrenched; but it may well be possible in future critical taxonomic studies to define and redefine 'variety' in such a way that its meaning will be clarified.

The author is grateful to Prof. J. S. Turner, Dr H. Godwin, Dr W. B. Turrill, Dr D. G. Catcheside and Mr A. J. Wilmott for help and advice.

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THE MERTON CATALOGUE

A LIST OF CHROMOSOME NUMBERS OF BRITISH PLANTS

SUPPLEMENT No. 1

By J. P. RUTLAND

John Innes Horticultural Institution, Merton, London

(With 19 figures in the text)

DICOTYLEDONS

RANUNCULACEAE

6. RANUNCULUS		
Ficaria L.	—*	(Fig. 1)

CRUCIFERAE

29. NASTURTIUM		
palustre DC	32	Scheerer 1939

CISTACEAE

57. HELIANTHEMUM		
guttatum Mill.	20†	Bowden 1940
polifolium Mill.	20	,,
canum Baumg.	22	,,

PORTULACACEAE

75. CLAYTONIA		
perfoliata Donn	36	Rutland num.nov.

TAMARICACEAE

77. TAMARIX		
gallica L.	24	Bowden 1924

GERANIACEAE

88. OXALIS		
corniculata L.	24	Rutland num.nov.

RHAMNACEAE

92. RHAMNUS		
Frangula L.	20	Rutland num.nov.

LEGUMINOSAE

99. TRIGONELLA		
ornithopodioides DC.	18	Rutland num.nov.
100. MEDICAGO		
falcata L.	16, 32‡	Ledingham 1940
101. MELILOTUS		
altissima Thuill.	16	Scheerer 1939
		Wipf 1939
102. TRIFOLIUM		
resupinatum L.	14§	Wipf 1939
agrarium L.	14	,,
dubium Sibth.	28	,,
104. LOTUS		
angustissimus L.	12‖	Rutland num.nov.
tenuis Waldst. & Kit.	12	Dawson 1941 (Native material)
hispidus Desf.	24	Rutland num.nov.

CRASSULACEAE

134. SEDUM		
rosea Scop.	22	Levan 1933
rosea var. *elongatum*	22	Tayohuko & Matsuura 1939
135. SEMPERVIVUM		
tectorum L.	72	Rutland num.nov.

HALORAGACEAE

138. MYRIOPHYLLUM		
alternifolium DC.	14	Scheerer 1939

ONAGRACEAE

142. EPILOBIUM		
palustre L.	36	Rutland num.nov.

UMBELLIFERAE

153. SMYRNIUM		
Olusatrum L.	22	Rutland num.nov. (Fig. 3)
156. APIUM		
inundatum Reichb. f.	22	Scheerer 1939
nodiflorum Reichb. f.	22	Rutland num.nov. (Fig. 2)
158A. PETROSELINUM		
crispum Nym.	22	Rutland num.nov.
172. OENANTHE		
silaifolia Bieb.	22	Rutland num.nov.

CAPRIFOLIACEAE

193. LONICERA		
Periclymenum L.	18	Rutland num.nov.

* The Mediterranean variety *calthaefolius* had $2n=48$ (Rutland). (Material kindly supplied by Prof. P. A. Buxton.)
† Chiarugi (1925) gives $2n=48$.
‡ Tetraploid previously reported by Tschechow (1933).
§ Bleier (1925) is probably wrong in reporting $2n=16$.
‖ Tschechow & Kartashowa give $2n=12$ and 24; but Dawson (1941) from a study of numerous examples regards this species as diploid only.

COMPOSITAE

204. SOLIDAGO *Virgaurea* L.	18	Scheerer 1939
207. ERIGERON *acris* L.	18	Rutland num.nov.
211. GNAPHALIUM *supinum* L.	28	Rutland num.nov. (Fig. 6)
212. INULA *Helenium* L.	20*	Rutland unpub. (Fig. 8)
crithmoides L.	18	Rutland num.nov. (Fig. 7)
217. DIOTIS *maritima* Hook.	18	Rutland num.nov. (Fig. 5)
220. MATRICARIA *suaveolens* Buch.	18	Rutland num.nov. (Fig. 4)
222. TANACETUM *vulgare* L.	18	Rosenberg 1905 Rutland unpub.
225. PETASITES *albus* Gaertn.	60	*Scheerer 1939
227. SENECIO *integrifolius* Clairv.	48	Rutland num.nov. (Fig. 9)
247. SONCHUS *oleracēus* L. em. Hill	32†	Rutland unpub.
asper Hill	18	Rutland num.nov.

CAMPANULACEAE

254. CAMPANULA *patula* L.	20	Rutland num.nov.
255. LEGOUSIA *hybrida* (L.) Del.	20	Rutland num.nov.

VACCINIACEAE

256. VACCINIUM *Myrtillus* L.	24	Rutland num.nov.

ERICACEAE

262. ERICA *mediterranea* L.	—‡	

APOCYNACEAE

283. VINCA *major* L.	92§	Pannocchia-Laj 1938 Bowden 1940 Rutland unpub.
minor L. [and two colour varieties]	46§	Pannocchia-Laj 1938 Bowden 1940 Rutland unpub.

GENTIANACEAE

288. GENTIANA *Pneumonanthe*	26	Scheerer 1939
290. NYMPHOIDES *peltatum* Britton & Rendle	54	Scheerer 1939

SCROPHULARIACEAE

314. SCROPHULARIA *nodosa* L.	36	Scheerer 1939
316. LIMOSELLA *aquatica* L.	40‖	Blackburn & Vachell 1939
319. VERONICA *humifusa* Dickson	14	Rutland num.nov.
scutellata L.	18	Scheerer 1939

LABIATEAE

332. ORIGANUM *vulgare* L.	30	Rutland num.nov. (Fig. 14)
334. CLINOPODIUM *vulgare* L.	20	Scheerer 1939
338. NEPETA *Cataria* L.	36¶	Sugiura 1940
hederacea Trev.	18	,,
hederacea Trev.	36	Rutland num.nov. (Fig. 13)
342. MARRUBIUM *vulgare* L.	34	Rutland num.nov.
343. STACHYS *sylvatica* L.	*c.* 66	Scheerer 1939
345. LEONURUS *Cardiaca* L.	18	Rutland num.nov. (Fig. 10)
347. BALLOTA *nigra* L.	22	Rutland num.nov. (Fig. 11)
348. TEUCRIUM *Scorodonia* L.	34	Rutland num.nov.
349. AJUGA *Chamaepitys* Schreb.	28	Rutland num.nov. (Fig. 12)

ILLECEBRACEAE

353. HERNIARIA *glabra* L.	18	Scheerer 1939

POLYGONACEAE

366. RUMEX *Acetosella* L. var. *tenuifolia*	28**	Löve 1940

EUPHORBIACEAE

373. EUPHORBIA *Cyparissias* L.	20	Rutland num.nov.
exigua L.	24	,,
Lathyris L.	20	Bowden 1940

SALICACEAE

389. POPULUS *nigra* L. var. *italica*	38	Suto, cit. Matsuura 1939

MONOCOTYLEDONS

ORCHIDACEAE

399. NEOTTIA *Nidus-avis* Rich.	36††	Barber 1941
404. CEPHALANTHERA *grandiflora* Gray	36	Barber 1941
405. EPIPACTIS *latifolia* All.	38	Barber 1941
406. ANACAMPTIS *pyramidalis* Rich.	36	Barber 1941
409. ACERAS *anthropophora* Br.	42	Barber 1941
410. OPHRYS *muscifera* Huds.	36‡‡	Barber 1941
apifera Huds.	36	Barber 1941

LILIACEAE

445. PARIS *quadrifolia* L.	20§§	Darlington and La Cour

NAIADACEAE

462. POTAMOGETON *crispus* L.	*c.* 52	Scheerer 1939
pectinatus L.	*c.* 78	,,
perfoliatus L.	*c.* 52‖‖	,,

* Confirms Tongiorgi (1935).
† Marchal (1920) gives also $2n=16$.
‡ *E. carnea*, the continental form of this species, has $2n=24$ (Hagerup, 1928).
§ Schürhoff & Müller (1937) give for *major* $2n=16$, for *minor* $2n=32$. Both these counts must be wrong
‖ Svensson (1928) gives $2n=36$.
¶ Bushnell (1936) gives $2n=32$; probably an error.
** Ono (1930) gives $2n=42$.
†† Agrees with Russian material.
‡‡ Senianinova (1925) gives $2n=22$–24.
§§ In the former list wrongly given as 10.
‖‖ Wisniewska (1931) gives $2n=c.\ 48$.

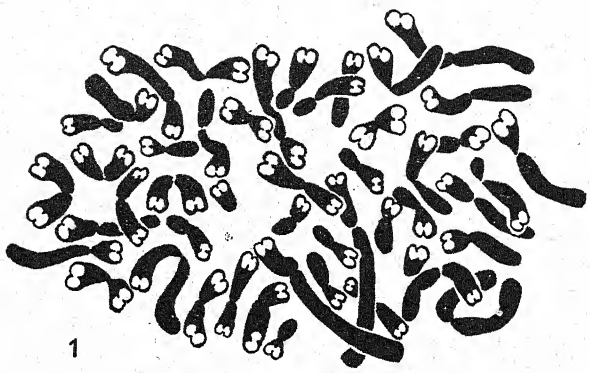
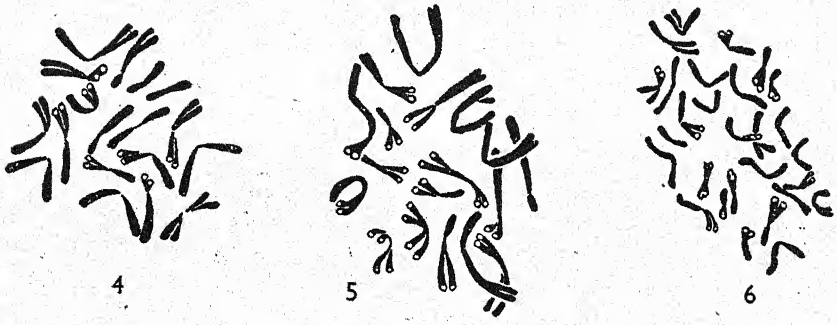
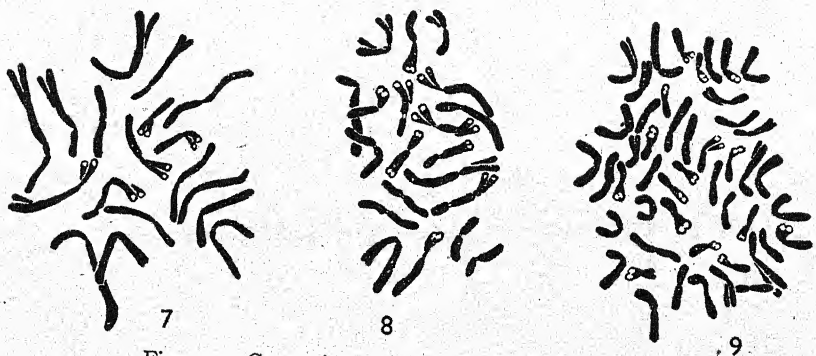


Fig. 1. *R. Ficaria*, $x=6$. (See p. 210.)

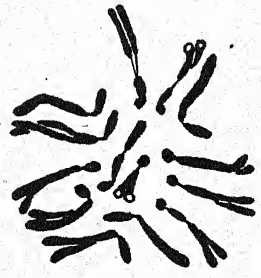
Figs. 2–3. Umbelliferae, $x=11$. (See p. 210.)

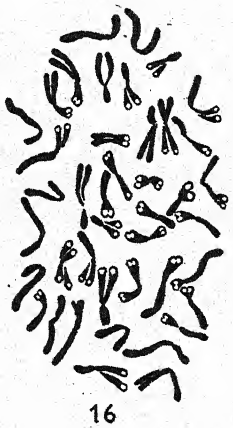


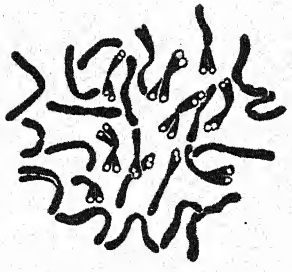


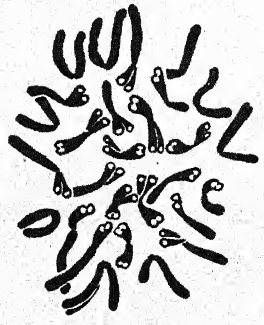
Figs. 4–9. Compositae, $x=7$, 9, 10, 12. (See p. 211.)

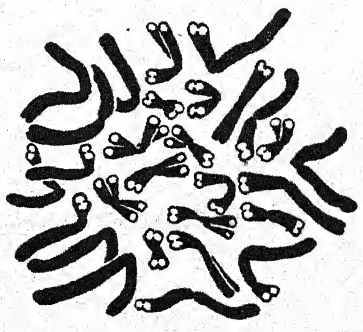
Figs. 10–14. Labiateae, $x = 5, 7, 9, 11$. (See p. 211.)











Figs. 15–19. Gramineae, $x = 7$. (See p. 214.)

CYPERACEAE

471. ERIOPHORUM		
polystachyon L.	58	Håkansson 1928
476. CAREX		
flava L.	60	Wahl 1940
canescens L. var. *disjuncta*	54	,,
diandra Schrank.	60	Heilborn 1939
paradoxa Willd.	64	,,

GRAMINEAE

489. POLYPOGON		
littoralis Sm.	28	Rutland num.nov. (Fig. 18)
491. GASTRIDIUM		
ventricosum Schinz & Thell.	14	Rutland num.nov. (Fig. 15)
500. AVENA		
pubescens Huds.	14*	Carson & Fyfe unpub.
501. ARRHENATHERUM		
tuberosum Schultz	28	Rutland num.nov. (Fig. 19)
514A. PUCCINELLIA		
fasciculata Bickn.	28	Rutland num.nov. (Fig. 17)
rupestris Fern. & Weath.	42	Rutland num.nov. (Fig. 16)
515. FESTUCA		
(*Vulpia*) *ambigua* Le Gall.	28	Maude 1940
516. BROMUS		
secalinus L.	14†	Nielsen 1939
commutatus Schrad.	56	,,

* Kattermann (1934) gives $2n=16$ (see Tischler 1936).
† A tetraploid, $2n=28$, previously reported by Avdulov (1931) and Nakajima (1931).

Notes. (1) My new counts here recorded are all from British material.

(2) All figures are magnified 3000 diameters from Flemming—gentian violet preparations.

ACKNOWLEDGEMENTS

I am indebted to Mr E. M. Marsden Jones, Mr H. N. Barber and Miss B. Schafer for many plants, and to Dr W. B. Turrill for assistance with the nomenclature.

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The references to earlier work given in the footnotes will be found in the Merton Catalogue.

REVIEW

Stratigraphie und Waldgeschichte des Wauwilermooses. By H. HÄRRI. 9×6 in. Pp. 104, 58 text-figs. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich, Heft 17. Bern: Hans Hüber, 1940. Price 7.50 Swiss frs.

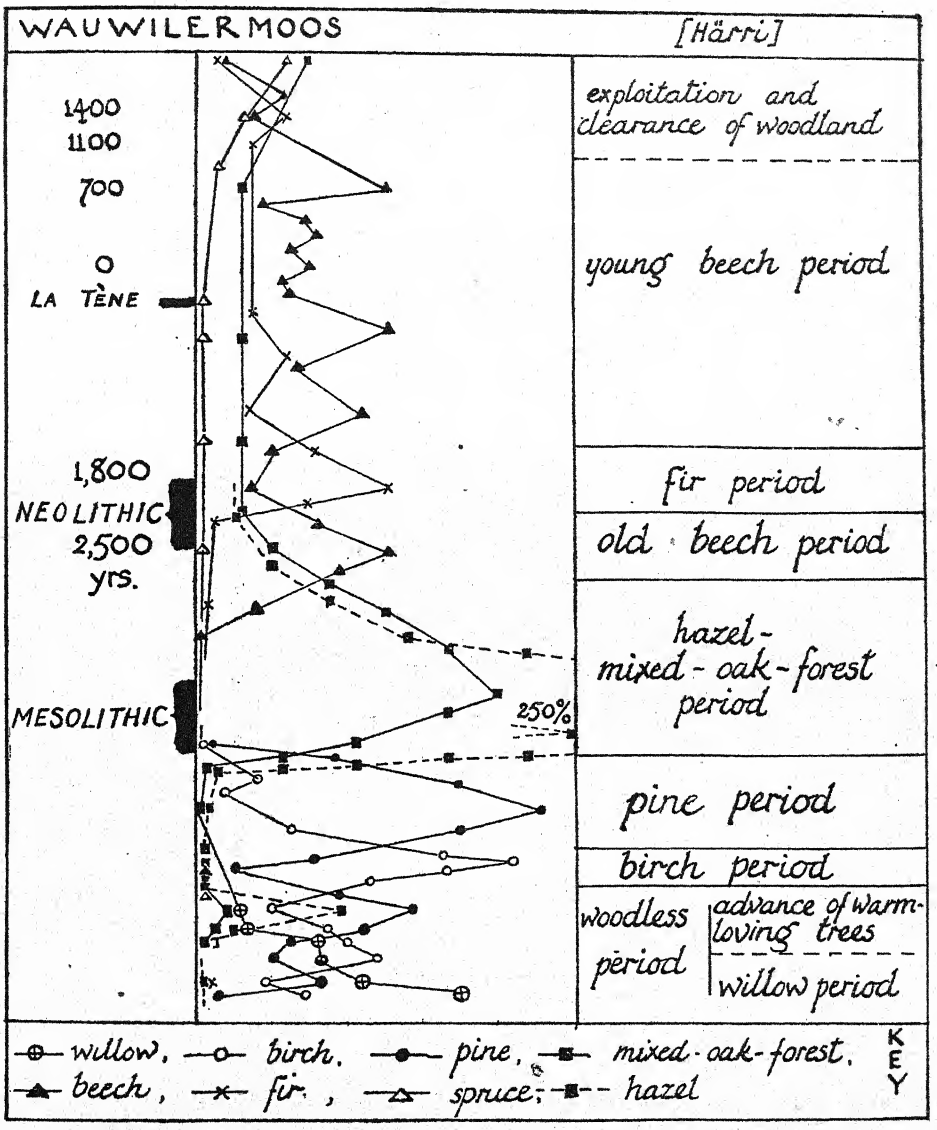

Fig. 1. Schematic correlation diagram for forest development at Wauwilermoos, near Lucerne, Switzerland, copied from Härri.

The Wauwilermoos has developed from a lake held up by a moraine of the last glaciation. It lies at an altitude of about 500 m., a few miles north-west of the Sempachersee, near Lucerne. Following its drainage extensive investigations revealed occupation sites of different archaeological periods. M. Härri has determined the stratigraphy of the lake- and bog-deposits by means of borings, and has made painstaking pollen-analyses at several points.

Many mesolithic and late neolithic horizons have been successfully brought into relation with a detailed and consistent picture of forest development, together with a single horizon of La Tène age. A copy of the author's generalized diagram is given in the accompanying Fig. 1, and from this the character of his results will be clear.

In the early forest-free period it is striking that after the willow period there is a stage in which pine replaces birch as the chief tree pollen and rather large amounts of hazel and the warmth-loving trees are present: in the following birch period the non-tree pollen remains low, but these more exigeant tree species are absent, and they do not return in large amounts until the opening of the hazel-mixed-oak-forest period. The temporary advance of the warmth-demanding trees is considered by the author as not due to the incorporation of pollen from older deposits, but as representing a short stage of improved climatic conditions. Similar effects are present in East Anglian deposits, and it will be recalled that in north-west Europe a similar fluctuation is referred to as the Allerød period, although in its typical form it is characterized there by solifluction effects on the stratigraphy which are absent in the Swiss lake deposits now described.

It is remarkable that up to the opening of the mixed-oak-forest stage the forest periods should be so similar in Switzerland and in north-west Europe, whilst after this stage the Swiss forest history, with phases of dominant *Fagus*, *Abies* and abundant *Picea*, is extremely different from our own. It may be that in the early periods climate was very much more uniform across Europe than it is now. Alternatively, it may be that the severity of low temperatures so restricted vegetation that it was similar over a range of topography and latitude, which under the later and milder conditions gave rise to a complex pattern of different woodland types.

The author attempts no correlation of his forest periods with those outside Switzerland, but one would suppose the hazel-mixed-oak-forest to correspond with the (Boreal) zones V and VI for England. If so, it becomes difficult to see why the author should say that this period is now regarded as wet, and on that account refuse a climatic basis for the rapid lowering in lake level at this time.

Another point of general interest to pollen-analysts lies in the difference which M. Härri can show between the diagrams of boring sites at different distances from the margin of the lake. *Fagus* is more strongly represented in the central sites and *Abies* in the marginal ones. This, one would think, points to the fact that *Fagus* must have been the more generally widespread of the two trees, and hence was better represented in the general pollen-rain falling in the lake centre: *Abies* must have been more localized near the lake and better represented therefore in its marginal deposits. The author does in fact recognize that his evidence, alone and considered with that of other Swiss investigators such as Lüdi and Keller, indicates a former considerable variation of forest cover from place to place, in response to differences of topography, soil, altitude, etc., just as at the present time.

It is of interest to the reviewer that a brief account is here given of the occurrence of peat 'nodules' (Torfknollen) in the layer just below the drained and mouldered surface of reclaimed peat land. These nodules are also found in the fen peat of East Anglia. They are smooth hard bodies of peat penetrated by fungal filaments and filamentous bacteria, and they are covered by a white weft of this material. They are sharply separate from the surrounding peat, so that they lie isolated from it in smooth cavities. They are certainly not faeces, fossil fruits nor even casts, and their formation seems more likely due to phenomena homologous with those of fungal fairy-rings, but taking place in three dimensions instead of two.

Throughout the account M. Härri is extremely cautious and unspeculative, and at times one wishes it were possible to discover more of his ideas as to correlation with other Swiss and other European forest-historical periods. All the same there is no doubt that his work brings us substantially nearer to the objective of making such correlations.

H. GODWIN

STUDIES IN ZYMASIS

VIII. THE DISCOVERY AND INVESTIGATION OF AEROBIC HCN ZYMASIS IN APPLES TREATED WITH HYDROGEN CYANIDE; AND COMPARISONS WITH OTHER FORMS OF ZYMASIS

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(With 5 figures in the text)

INTRODUCTION

THE term *zymasis* was first used as an abbreviation for *zymasic metabolism in the cells of the higher plants* by Thomas (1925) at a time when the notion of cellular fermentations still suggested faintly the presence of micro-organisms. The essential feature of the process is that ethyl alcohol, with, possibly, its precursor acetaldehyde, should accumulate in the cells. It is a form of anaerobic glycolysis, directed by the zymase complex, which has proceeded beyond the stage of carboxylase cleavage. Accordingly, the carbon dioxide produced by the decarboxylation of pyruvic acid is also reckoned among the products of zymasis.

Two types of method have been used for detecting and measuring zymasis, and for determining its metabolic significance. The identification of such ethyl alcohol and acetaldehyde as accumulate, and their measurement, either severally or in the sum as an alcohol number (see p. 220), yields unambiguous evidence of the occurrence and extent of zymasis (Thomas, 1925; Fidler, 1934). In several researches the occurrence of zymasis has virtually been inferred from changes found in the rates of CO_2 output and oxygen uptake, and, consequently, in the respiratory quotient (e.g. see Stich, 1891; Blackman, 1928; Genevois, 1927; van Raalte, 1937; James & Hora, 1940). In the present investigation these two types of method have been used in conjunction.

Anaerobic zymasis occurs vigorously in the apple (Thomas, 1925), as it does in many other tissues (see Kostytschew, 1927), living precariously in the complete absence of oxygen. The work of Stich suggests that all these tissues would probably show *low oxygen zymasis* when placed in gas mixtures containing oxygen at a concentration below a certain critical value, which has been called the extinction point of anaerobic respiration. For healthy apples this value may be lower than 3 % oxygen (Thomas & Fidler, 1933). The normal aerobic metabolism of apple cells, or, more precisely, the normal activity of those oxidation enzymes that are linked therein with the zymase complex, is lively enough to suppress zymasis completely,

when the atmosphere around the fruit contains more than this critical value of oxygen.

We note that it is most unusual to find more than very small amounts of ethyl alcohol and acetaldehyde in healthy plant tissue surrounded with pure air; but in damaged fruits *injury zymasis* may take place (Thomas, 1931), and in apples (Fidler, 1933*a*) and many other fleshy fruits *senescence zymasis* occurs as they age in air. The view we hold is that after injury and during senescence the activity of oxidative processes becomes reduced in apple cells to levels less than those which would be reached in healthy young fruit placed in atmospheres containing less than 3 % oxygen.

In all the forms of zymasis that we have mentioned acetaldehyde as well as ethyl alcohol accumulates in the apple, but the maximum concentration of acetaldehyde developed is very low compared with that of ethyl alcohol.

If the depression below a certain critical level of oxidative activity, relative to intracellular zymase activity, is the sole necessary antecedent for the occurrence of zymasis in such tissues as show anaerobic zymasis, ethyl alcohol with, possibly, acetaldehyde should accumulate aerobically in plant cells treated with substances that differentially inhibit respiratory enzymes. Such inhibition by carbon dioxide was postulated by Thomas (1925) who discovered *CO_2 zymasis* as a process occurring in apples placed in gas mixtures containing from 5 to 60 % oxygen and high concentrations of carbon dioxide (usually greater than 30 %). In spite of the presence of abundant oxygen in the external atmosphere, respiratory metabolism resembled that which it would have shown had the external oxygen concentration been less than 3 %. An interesting point was that the operation of the zymase complex was modified inasmuch as far higher concentrations of acetaldehyde were developed during CO_2 zymasis than during parallel experiments on anaerobic zymasis. This suggested that there had been differential inhibition not only of oxidation enzymes concerned with aerobic metabolism, but also of aldehyde oxido-reductase within the zymase complex.

The extensive and intensive researches in the middle 1920's on the inhibitory influences exerted by cyanides and other poisons on the oxidative metabolism (including the Pasteur reaction) of healthy and cancerous animal tissues (Warburg, 1926), and of yeast cells (Meyerhof, 1925), and on the activities of oxidation enzymes that contain iron-porphyrin compounds (see, e.g. Dixon, 1929), was an obvious lead to similar investigations on plant tissues and oxidation enzymes extracted from them. Thomas (1930) discovered that ethyl alcohol and acetaldehyde accumulate in apples placed in air containing the vapour either of HCN or of H_2S, i.e. poisons which had been shown to inhibit differentially certain oxidation enzymes (e.g. cytochrome oxidase), and that the respiratory quotient rose above unity. Other tissues that produced these substances anaerobically behaved similarly. In apples the acetaldehyde concentrations developed were even higher than those found after CO_2 zymasis. The phenomena of HCN zymasis and H_2S zymasis in plants had been discovered; and it was decided to make a thorough investigation of HCN zymasis in the apple in order to elucidate the metabolic significance of this process.

In the present and the next paper (pp. 240–261) we record and discuss the results of this research.

1. Experimental methods and the expression of results

Plant material and experimental procedure

Several varieties of apple have been used, because in each season the experiments lasted longer than the storage life, in Newcastle, of a single variety. With the exception of Newton Wonder and Bramley's Seedling, which came from an orchard in Kent, imported fruit was bought at Newcastle as required.

Most of the Newcastle experiments were carried out at fluctuating laboratory temperatures. Experiments on Sturmer Pippin and Newtown Pippin were performed in a room kept at 12° C. in the Low Temperature Research Station, Cambridge. We are most grateful to Dr Franklin Kidd for the facilities provided.

Gas streams—air in the aerobic experiments, and cylinder nitrogen, from which the oxygen impurity was removed, in the anaerobic experiments—containing different concentrations of HCN vapour, or, in the control experiments, free from this poison, were passed over comparable samples of apples placed in desiccators fitted with inlets and outlets, and issued into small volumes of acidified phloroglucinol solutions. When zymasis is proceeding vigorously a precipitate appears in this reagent (p. 229), which is therefore a useful indicator.

Except in the experiments on Sturmer Pippin, hydrogen cyanide was generated by bubbling a gas stream through neutral solutions of potassium cyanide (B.D.H. Analar). In our earlier experiments these solutions were placed inside the desiccators, and in the later ones outside. In the experiments on Sturmer Pippin higher cyanide concentrations were obtained by passing air through acidified solutions of potassium cyanide. Our attempts to maintain constant concentrations of HCN vapour over long periods have not been successful.

The experiments fall into two groups: those in which apples have been continuously acted on by air (or nitrogen) containing HCN vapour, and those in which such treatment has gone on for a period, and subsequently pure air has been passed over the fruit. In both groups, by varying the external concentration of cyanide, we have obtained data for the rates of cyanide accumulation under different conditions, and for the relationship between these rates and the rates of zymasis. Experiments in the second group have provided information about the fate under aerobic conditions of cyanide, ethyl alcohol, and acetaldehyde, that accumulated during the period of HCN uptake, and also about the physiological state of the tissue at the end of this period. Care has been taken not to confuse HCN zymasis with the injury zymasis that may result from the disorganization of cells poisoned by cyanide.

In all the experiments one or more apples were removed from each desiccator at regular intervals, and the hydrogen cyanide, ethyl alcohol, and acetaldehyde contained in them were expelled by steam distillation. The distillates were analysed.

Methods of analysis

(*a*) *Hydrogen cyanide.* It is the internal and not the external concentration of cyanide that determines metabolic events in living cells. Consequently the HCN concentration in the gas passing over apples has not been measured. By titrating with silver nitrate a portion of the steam distillate, we have obtained a number representing the relative internal concentration of cyanide. We do not assert that this number is an exact measure of all the cyanide absorbed, for it is probable that HCN may be consumed in a number of ways by living cells, but in general we have found that its value increased fairly regularly with the continuous exposure of fruit to gas streams containing cyanide.

(*b*) *Alcohol number, acetaldehyde, and ethyl alcohol.* First, the alcohol number, which represents the sum of ethyl alcohol and acetaldehyde, was determined by a method described elsewhere (Fidler, 1934). Clausen's and Ripper's methods were used for estimating acetaldehyde. In the presence of cyanide the end-point is hard to judge, especially with Clausen's method, and our results for acetaldehyde may be somewhat higher than the true values. Consequently, the values recorded for ethyl alcohol, which are obtained by difference, will be lower than the true values. None of the conclusions we shall draw are affected by these errors. It is important to note that the presence of cyanide is not a disturbing factor in the determination of the alcohol number, since it is a simple matter to estimate and allow for any HCN that may be present in the final distillate that is titrated with standard alkali.

Expression of results

In this and in papers to come we shall take into account not only the amounts of cyanide and products of zymasis that accumulate over certain periods, but also the rates of accumulation. The following symbols will be used:

For accumulation:

y mg. cyanide number in 100 g. fresh-weight tissue.
z mg. alcohol number in 100 g. fresh-weight tissue.
d mg. acetaldehyde in 100 g. fresh-weight tissue.
e mg. ethyl alcohol in 100 g. fresh-weight tissue.

For rates of accumulation:

Y average daily increase in y.
Z average daily increase in z.
D average daily increase in d.
E average daily increase in e.

For example, an increase from 10 to 70 in the alcohol number (z) over a period of 3 days, while the cyanide number (y) increased from 0·1 to 3·1, would mean a rate of cyanide accumulation (Y) of 1 and of zymasis (Z) of 20. It should be noted that the alcohol number provides an unambiguous measure of the amount of zymasis, and Z of the rate of this process.

As zymasis in apples is always to some extent arrested at the acetaldehyde stage, the magnitudes d and D relative to e and E are informative, as is also the ratio D/Z.

To avoid ambiguity clarifying symbols will sometimes be suffixed to the letters defined above. Thus Z_{N_2} will mean the rate of anaerobic zymasis, $Z_{Air+HCN}$ the rate of aerobic HCN zymasis.

2. The accumulation of hydrogen cyanide in apples

The cyanide number (y) for apples kept continuously in air has usually been less than 0·1. This number may increase slightly under certain conditions, possibly owing to the hydrolysis of the cyanogenetic glucoside contained in the pips. The drifts of the values given as italicized numbers in Tables 1 and 2, and as points on the curves in Figs. 1*a*, 2 and 3*a*, show that in all our experiments the cyanide number increased at once and steadily upon exposing apples to air containing the vapour of hydrogen cyanide.

These charted and tabulated records indicate that when neutral solutions were aerated (i.e. in all experiments except XXV–XXIX), the rate of cyanide accumulation was determined by the concentration of potassium cyanide. This is also shown by the average daily rates (Y), calculated for certain selected periods, which are recorded in Table 4. Thus for Dunn's Favourite (XI, XII, XIII) during the first eight days the Y values were 0·03, 0·08 and 0·2, with external cyanide concentrations of 0·5, 2 and 5 % respectively; and for Newton Wonder (VIII and X, and III and V) and Y values over 3 days were 0·17 and 2·3 with external concentrations of 0·05 and 5 % respectively. It will be observed that for a given external concentration in the experiments on Newton Wonder this rate was greater than in those on Dunn's Favourite. The results obtained in many of our experiments indicate, however, that not only varietal differences among apples, but physiological age and health in a given variety may affect this rate, and the subsequent behaviour of the fruit.[1]

When neutral solutions were aerated the maximum possible rate of cyanide accumulation was never approached. Although in XXV–XXIX on Sturmer Pippin the temperature was several degrees lower than in any of our other experiments, the use of *acidified* solutions of potassium cyanide resulted in a great increase in Y (Fig. 2 and Table 4). Thus for the lowest external concentration, viz. 1 % in XXIX, a y value of 34 had been reached at an average Y rate of 5.

The results of these experiments on Sturmer Pippin were surprising in that in uninjured fruit neither Y nor y showed any tendency to increase with increasing external cyanide concentration. As the experiments were not carried out simultaneously it remains possible that the cyanide at the lower concentration entered the fruit more rapidly in February than it would have done 2 months earlier. This

[1] We have not attempted to determine whether magnitudes such as the rate of HCN accumulation, the rate of zymasis for a given Y value, the D/Z value under defined conditions, proneness to aldehyde poisoning, can be correlated with keeping qualities of fruit or some other property of commercial importance.

Tables 1 and 2. *Variations at laboratory temperatures in January 1932 in the cyanide number (y), alcohol number (z), and acetaldehyde (d) and ethyl alcohol (e) concentrations, as defined on p. 220 in Newton Wonder while under cyanide treatment (numbers in italics), and subsequently in pure air*

Table 1 (Exps. I–V). During cyanide treatment exposure was to air which had passed through neutral 5 % KCN

Days from 9. i. 32	I				II				III				IV				V			
	y	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*
Air values at outset	*0·1*	*7·5*	*0·5*	*7*																
1	*1*	*28*	*11*	*17*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	0·5	31	6	25	*4·5*	*65*	*26*	*39*	.	.	.	.	.	.	.	.	.	.	.	.
3	1	48	4	44	5·5	121	41	80	*7·0*	*90*	*32*	*58*	.	.	.	.	.	.	.	.
4	0·5	27	3	24	6·5	130	31	99	*7·5S*	97	34	63	*13S*	*80*	*38*	*42*	.	.	.	.
5	0·5	24	1	23	5·0*S*	92	29	63	6·5	93	34	59	10	112	54	58	*12S*	*81*	*37*	*44*
6	0·5	22	4	18	5·0	98	19	79	5·5	124	38	86	9·5	102	39	63	12	104	47	57
7	.	.	.	.	5·0	113	28	85	5·5	157	66	93	11*B*	113	47	66	9·0*B*	82	32	50
8	.	.	.	.	.	.	.	.	5·5	154	34	120	6·5	68	36	32	9·0	92	39	53
9	.	.	.	.	.	.	.	.	.	.	.	.	8·5	90	34	56	10	135	50	85
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9·5.	103	48	55

(i) *S*=apple shows surface injury, *B*=apple completely brown.
(ii) The average for all these experiments of the *D*/*Z* ratio is about 1/2·5, indicating that HCN retarded the reduction of acetaldehyde.

Table 2 (Exps. VI–X). During cyanide treatment exposure was to air which had passed through neutral 0·05 % KCN

Days from 6. ii. 32	VI				VII				VIII				IX				X			
	y	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*	*y*	*z*	*d*	*e*
Air values at outset	*0·1*	*7·5*	*0·5*	*7*																
1	*0·3*	*14*	*4*	*10*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	0·4	55	3	52	*0·9*	*34*	*7*	*28*	.	.	.	.	.	.	.	.	.	.	.	.
3	0·3	13·5	0·5	13	0·7	49	7	42	*0·6*	*19*	*2*	*17*	.	.	.	.	.	.	.	.
4	0·1	11·5	0·5	11	0·6	46	4	42	0·4	30	2	28	*0·8*	*51*	*9*	*42*	.	.	.	.
5	0·1	21	1	20	0·5	32	2	30	0·5	52	3	49	0·7	57	3	54	*1*	*57*	*6*	*51*
6	0·1	18	1	17	0·6	31	2	29	0·4	46	2	44	0·7	36	3	33	0·9	41	3	38
7	.	.	.	.	0·5	26	3	23	0·2	34	2	32	0·7	38	3	35	1·3	52	3	49
8	.	.	.	.	.	.	.	.	0·3	27	1	26	0·5	36	2	34	0·8	32	2	30
9	.	.	.	.	.	.	.	.	.	.	.	.	0·7	29	2	27	0·8	36	3	33
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1·2	85	4	81

The average *D*/*Z* ratio while HCN was still accumulating was 1/6.

would mean that Y tends to increase as apples grow older. Nevertheless, it is probable that during the first week of these experiments, Y was not wholly determined by the external cyanide concentration but in part by the capacity of each apple to absorb hydrogen cyanide. In all the experiments some of the apples were

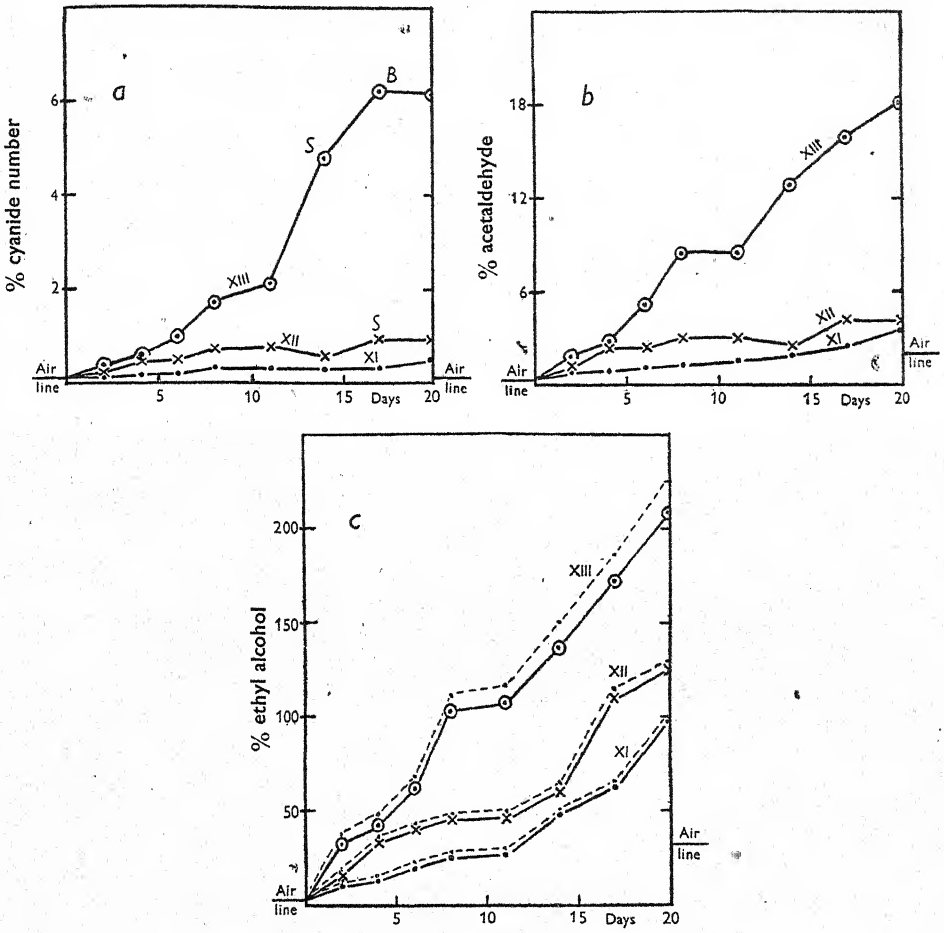

Fig. 1 *a*, *b* and *c*. The aerobic accumulation in May and June 1932, at room temperature, in mg./100 g. fresh-weight Dunn's Favourite (XI, XII, XIII) treated with HCN, of the cyanide number (*a*), acetaldehyde (*b*), and ethyl alcohol (*c*). Changes in the alcohol number are also shown in *c* by interrupted lines. In XI the apples were continuously exposed to air that had previously passed through 0·5 % KCN in an outside container; in XII 2 % KCN was used, and in XIII 5 % KCN. The first sign of injury is indicated by *S*, and extensive browning by *B*.

injured before the end of the week. In XXV the injuries were severe, and the doubling of the Y value in the second week suggests that properties of the living apple cells played a part in limiting the rate of cyanide accumulation during the first week. Injury as an antecedent to accelerated accumulation was also observed during the last 2 days of XVII on Dunn's Favourite (Table 4).

In our experiments on all varieties, y steadily increased while apples were exposed to HCN vapour, and no evidence of saturation was obtained. We have not yet succeeded in measuring HCN zymasis in uninjured fruit under a constant cyanide number, because when such fruit containing hydrogen cyanide was replaced in air the y values declined (Table 1, III–V; Fig. 3*a*, XIV–XVI).

3. THE OCCURRENCE AND PROGRESS OF HCN ZYMASIS

Concomitant increases in the alcohol number (z) and cyanide number (y)

In our experiments with numerous varieties of apple (Newton Wonder, Bramley's Seedling, Dunn's Favourite, Cleopatra, Granny Smith, Newtown Pippin, Beauty of Bath) we have found that while cyanide is accumulating in fruit exposed to air containing HCN vapour the alcohol number (z) increases more than it does

Table 3. *Exp. XXII on Granny Smith in June and July* 1933.
(*Description as for Table* 1)

Conditions	Days from 29. vii. 33	y	z	d	e	Remarks
Pure air	3	—	7·2	0·7	6·5	
Air plus vapour from 5 % KCN	5	3	55	4·4	51	
	11	6·5	162	22	140	All apples sound. $D/Z = 1/8$.
Apples returned to pure air	15	5·1	169	27	142	One apple sound; two showed surface injury
	18	5·3	291	31	260	One apple sound; two showed surface injury
	21	4·7	194	22	172	One apple completely brown; three severely injured
	21	4·0	236	26	210	One apple slightly injured; two completely brown

in the control experiments. What we shall describe by the term HCN zymasis has resulted from the action exerted by HCN upon the aerobic metabolism of carbohydrates by the flesh tissue. In HCN zymasis acetaldehyde as well as ethyl alcohol accumulates, and an amount of CO_2 equivalent to the alcohol number is given off. Other processes yielding CO_2, e.g. persisting aerobic respiration, may take place simultaneously.

The evidence on which these contentions rest is contained in the tables and charts published in this and the following paper. It requires little explanation. For Dunn's Favourite the graphic records of Figs. 1 and 3, for Newton Wonder the drifts of the italicized numbers in Tables 1 and 2, for Granny Smith the values in Table 3, and for Sturmer Pippin the charts in Fig. 2 show, upon inspection, that zymasis occurred when each of these varieties was under cyanide treatment. In the tables, the alcohol numbers are seen to increase with time; and, in the figures,

the progress curves slope upwards, often steeply, from the air-lines. In nearly all the experiments reported in this paper, zymasis appeared to set in early and continued at a steady rate during the first few days. In a few experiments (e.g. XXIII

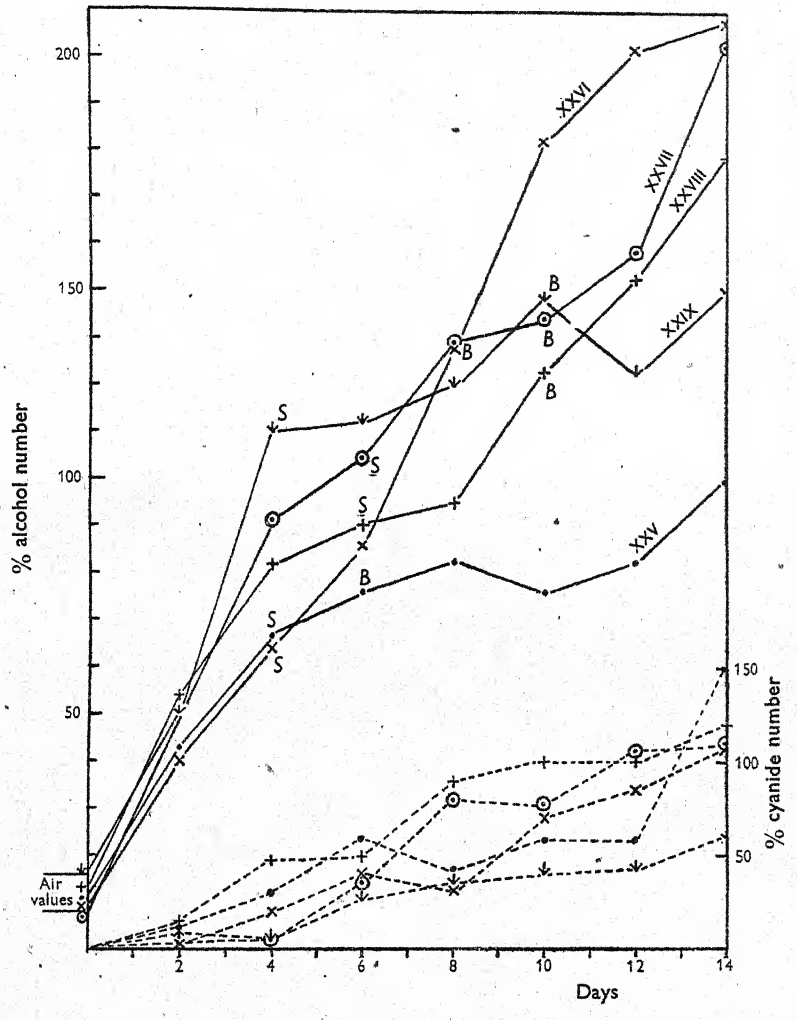

Fig. 2. Increases in the cyanide number (interrupted lines) and in the alcohol numbers of Sturmer Pippin (XXV–XXIX) treated at 12° C. with HCN. In the several experiments acidified solutions of KCN, of strengths recorded below, were aerated in outside containers. The first sign of injury is indicated by *S*, and extensive damage by *B*:

• 9. xii. 36, 40 % KCN, XXV. × 2. i. 37, 15 % KCN, XXVI. ⊙ 20. i. 37, 10 % KCN, XXVII.
+ 1. ii. 37, 3 % KCN, XXVIII. ↓ 15. ii. 37, 1 % KCN, XXIX.

on Newtown Pippin, see p. 246), in which very low external concentrations of cyanide were used, there have been indications of a lag between the first increase of the cyanide number and that of the alcohol number, suggesting that zymasis is not always immediately induced when cyanide is absorbed by plant cells.

Reduced oxidative activity as the probable cause of HCN *zymasis*

We claim that by demonstrating the phenomenon of HCN zymasis in plant tissues surrounded with air containing HCN vapour we have secured evidence that cyanide-sensitive oxidation enzymes participate in the normal aerobic oxidative metabolism of carbohydrates. Moreover, we maintain that HCN zymasis is actually induced as a result of the progressive inhibition of such enzymes by the absorbed cyanide, the conditions in the cells becoming by degrees akin to those prevailing in the absence of cyanide when the oxygen concentration is gradually reduced from 20 % to zero. The results obtained by measuring gaseous exchanges, particularly of the rates of oxygen uptake, which are discussed on pp. 243–251 in the next paper, have provided supporting evidence for these views.

We could go further were we certain that under all conditions the rate of zymase cleavage is not affected by the presence of cyanide in apple cells; for we then could infer that ethyl alcohol and acetaldehyde would not accumulate until oxidative activity had been depressed by cyanide at least as much as it would be when, in the absence of cyanide, there was only 3 % oxygen in the gas outside the fruit. Smaller depressions would be reflected in reduced rates of aerobic respiration, without accompanying zymasis.

General conclusions have to be worded cautiously, because the results we have obtained suggest that, during the course of many of our experiments, the activity of zymase was partially inhibited as a result of the presence of cyanide in apple cells (§ 8). Inasmuch as HCN zymasis occurred in our aerobic experiments even when apples received very strong doses of cyanide, we may conclude, however, that zymase in apple cells is much less prone to inhibition than are the enzymes concerned with normal aerobic oxidative metabolism. Zymase may be less exposed in the protoplasm to the penetrating cyanide than are oxidation enzymes, or its catalytically active surfaces may be less sensitive to cyanide poisoning.

It should be noted that we have not excluded the possibility that, under certain conditions, the rate of zymase-cleavage may be temporarily enhanced in living cells treated with HCN.

The rate of HCN *zymasis*

In all our experiments the initial rate of HCN zymasis (Z) appears to have been governed by the rate of cyanide accumulation (Y). It was the concentration of aerated neutral solutions that determined not only the value of Y (§ 3) but also that of Z. For Dunn's Favourite (Fig. 1 *a*, *b*) there is clear evidence of this in the different pitches of the corresponding progress curves for increases in the cyanide number and alcohol number. During the first 8 days (Table 4, XI, XII and XIII) average Y rates of 0·03, 0·08 and 0·21, led to average zymasis rates of 2·5, 5·5 and 12·9; and during the remainder of the experiment Y rates of 0·01, 0·03 and 0·37, to Z rates of 6, 6·8 and 9·8. In XII and XIII the rates remained fairly steady and injuries were not seen until a late stage. Experiment XVII (Table 4) is interesting because the rates of cyanide accumulation and zymasis were higher during the last 2 days than in any of the other experiments on Dunn's Favourite.

Our results show no regular proportion between the rate of zymasis and any cyanide value we have measured or calculated. The falls in the corresponding values of Z/Y in the final column of Table 4 indicate that although the rate of zymasis increased as the external concentration of cyanide increased, it did so relatively less rapidly than the rate of cyanide accumulation. This may be because the whole cell including the wall and vacuole absorbs hydrogen cyanide, and only a fraction of the absorbed cyanide brought about those depressions of oxidative activity that lead to zymasis. Furthermore, zymase cleavage may have been progressively retarded as the cyanide number increased, or the penetration of the poison became deeper.

Higher values of Y and Z were met with in parallel experiments on Newton Wonder (Table 4). During the first 3 days of VIII and X, with a Y rate of 0·17, the zymasis rate was 3·8; and in III and V the higher Y rate of 2·3 induced zymasis at a Z rate of 28. The numbers in the last column show that unit rate of cyanide accumulation was nearly twice as effective at the lower external concentration of cyanide.

It will be observed that during the last 2 days of III and V cyanide accumulation continued, but zymasis did not occur. At this stage the apples were severely injured and some were completely brown. Zymasis had begun in perfectly healthy fruit. It continued for a short time after the appearance of injury, but stopped with the death of cells. The behaviour of poisoned fruit was variable. In XXII on Granny Smith (Table 3) and XXIII on Newtown Pippin (p. 246) death occurred more slowly, and the rates of zymasis were high during the pre-mortem period of autolysis. New complexities had been added, seeing that injury zymasis may participate in the zymasic metabolism of tissues dying in the presence of cyanide.

Acidified solutions of potassium cyanide were aerated in XXV–XXIX on Sturmer Pippin, resulting in high rates of cyanide accumulation (Fig. 2 and Table 4). These rates did not vary with the external cyanide concentration (§ 2), and the recorded results show that during the first week, before the fruit was injured, the rates of zymasis were also independent of the external cyanide concentration. The pitches of the progress curves for increases in the alcohol number do not steepen with increasing external cyanide concentrations. The Z values (Table 4) range from 10 to 15·4 and show no regular increase with increasing external cyanide concentrations from 1 to 40 %. In these experiments, in contrast to the experiments described above, the Z/Y ratios did not decrease as the external cyanide concentration was increased. The italicized values for the first week are not widely divergent; possibly they would have been constant had larger samples been used for analysis, and had experimental methods been more refined. The conclusion to be drawn would then have been that the maximum rate of Y, and therefore of Z, was reached with the 1 % external solution, and that during the first week the activity of zymase in the apple cells was either not depressed at all or depressed to an equal degree in all the experiments. Had we used lower concentrations than 1 % it is probable that the values of Y, Z and Z/Y would have been governed by the external cyanide concentration as they were in the experiments on Newton Wonder and Dunn's Favourite.

Table 4. *The average rates of cyanide accumulation and of* HCN *zymasis in several experiments**

Exp. no.	Ext. conc. KCN %	Periods in days†	Increase in amounts during period		Average daily rates		Rate of HCN zymasis / Rate of cyanide accumulation i.e. Z/Y
			Cyanide number y	Alcohol number z	Increase in cyanide number Y	Increase in alcohol number‡ Z	
Dunn's Favourite							
XI	0·5	8	*0·3*	*20*	*0·03*	*2·5*	*80*
		12	0·2	71§	0·01	6	600
XII	2	8	*0·65*	*44*	*0·08*	*5·5*	*70*
		12	0·30	81	0·03	6·8	272
XIII	5	8	*1·7*	*103*	*0·21*	*12·9*	*61*
		12	4·5	118	0·37	9·8	26
XVII	5	4	0·85	46	0·21	11·6	55
		2	2·5	64	1·23	31·7	25
Newton Wonder							
VIII and X	0·05	3	*0·5*	*12*	*0·17*	*3·8*	*22*
		2	0·4	38	0·2	19	95
III and V	5	3	*7*	*83*	*2·3*	*28*	*12*
		2	5	Nil	2·5	Nil	Nil
Sturmer Pippin							
XXIX	1	7	*34*	*100*	*5*	*14*	*2·8*
		7	24	23	3	3·3	1·1
XXVIII	3	7	*69*	*80*	*10*	*11*	*1·1*
		7	52	77	7	11	1·6
XXVII	10	7	*58*	*108*	*8*	*15·4*	*1·9*
		7	51	76	7	11	1·6
XXVI	15	7	*36*	*99*	*5*	*14*	*2·8*
		7	72	91	10	13	1·3
XXV	40	7	*52*	*70*	*7*	*10*	*1·4*
		7	98	21	14	3	0·2

* XXV–XXIX were carried out at a constant temperature, viz. 12° C. The other experiments were performed under fluctuating room temperatures. XI, XII and XIII form a parallel set, as do III, V, VIII and X.

† In all the experiments the periods were successive, i.e. XI–XIII lasted 20 days, those on Newton Wonder 5 days, and on Sturmer Pippin 14 days.

‡ The number in this column represents the rate of HCN zymasis (p. 221).

§ Senescence zymasis or injury zymasis may have been responsible for some of the increase in the alcohol number during this period.

It was expected that the rate of HCN zymasis would have increased while cyanide was accumulating in the tissue, but we have not as yet in any of our experiments obtained clear evidence of this. Thus in XXV–XXIX on Sturmer Pippin the form of the curves in Fig. 2 is not obviously sigmoid. Rather it suggests that the rate of zymasis was fixed early on and maintained during the course of the first week, while the cyanide numbers rose to y values ranging between 34 and 69. In spite of these high values and the presence of products of zymasis only a few of the apples were injured during the first week. Later there was much damage seen, especially in XXV with the external concentration of 40 % cyanide. The progress curve for this experiment flattens after the first week, Z falls from 10 to 3, and Z/Y from 1·4 to 0·2; with the onset of injury the rate of cyanide accumulation increased while the rate of zymasis fell. Further evidence is thus provided that HCN zymasis is a phenomenon of living cells, and stops with their death.

4. The accumulation of acetaldehyde during HCN zymasis

The demonstration of acetaldehyde production

The production in apples undergoing HCN zymasis of a volatile aldehyde may be demonstrated by passing the gas issuing from them through an acidified solution of phloroglucinol (Thomas, 1925). In a day or two a precipitate of acetaldehyde phloroglucide will appear in the reagent, while none is formed in the control experiments. Acetaldehyde escapes as vapour from the flesh tissue, where it accumulates in the free state to such an extent that the cell sap may give (*a*) a red colour with Schiff's reagent, and (*b*) a blue colour with sodium nitroprusside and piperidine (Rimini's test).

The rate of accumulation (including escape) of acetaldehyde, and varying values of the ratio D/Z

The rate of zymasis (Z), as defined on p. 220, will be a measure of the rate of production of acetaldehyde, assuming that this substance is produced only by zymase cleavage, and that its sole metabolic fate is reduction to ethyl alcohol. Since in our experiments on zymasis, reported in this and in earlier papers, we have always found ethyl alcohol to be present with acetaldehyde, we infer that the rate of accumulation of acetaldehyde (D) has always been lower than the rate of its production. There is a tendency, however, in certain forms of zymasis for zymase cleavage to be arrested at the acetaldehyde stage. For Newton Wonder this is indicated by the values tabulated below for the maximum aldehyde number (d) and the average D/Z ratio in pure nitrogen or pure CO_2, high concentrations of CO_2 in the presence of oxygen, and during HCN zymasis. It is clear that HCN zymasis in this variety resembles CO_2 zymasis in that the reduction of the acetaldehyde produced tends to be arrested. Both forms differ unmistakably from anaerobic zymasis. The difference in the D/Z ratio for strong and weak cyanide shows that

retardation of aldehyde reduction becomes more marked as the cyanide number increases.

	Anaerobic zymasis	CO_2 zymasis	HCN zymasis	
			Strong cyanide	Weak cyanide
Maximum *d* value found	6	60	66	9
Average *D*/*Z*	1/51	1/3	1/2·5	1/6

The behaviour of other varieties during HCN zymasis was similar to that of Newton Wonder. Under weak cyanide treatment Dunn's Favourite (Fig. 1) showed a maximum *d* value of 13 and a *D*/*Z* ratio of 1/8; stronger cyanide dosing of Granny Smith (Table 3) produced a *d* value of 31 and the *D*/*Z* ratio was 1/8. Very high cyanide numbers were developed in Sturmer Pippin (Table 5), and the record *d* value of 162[1] was found, with *D*/*Z* ratios varying between 1/1 and 1/1·5; in most of the experiments more than 70 % of the acetaldehyde produced by zymase cleavage had accumulated. The rate of accumulation of acetaldehyde was greater than that of its reduction to ethyl alcohol.

As a possible cause of the partial arrest of zymasis at the acetaldehyde stage we dismiss fixation of the aldehyde by cyanide because the amount of HCN absorbed was not enough to account for the acetaldehyde value, except in the experiments

Table 5. *Injuries resulting from the accumulation of cyanide and products of aerobic* HCN *zymasis in Sturmer Pippin at* 12° C. (*cf. Table* 3)*; and average D*/*Z ratios*

Exp. no.	At first sign of injury				Final values after 14 days		
	Days	Cyanide number *y*	Alcohol number *z*	Acetaldehyde *d*	Alcohol number *z*	Acetaldehyde *d*	*D*/*Z* average for whole period
XXV	4	31	58	24	91	69	1/1·4
XXVI	4	20	58	40	190	162	1/1·2
XXVII	6	35	96	62	184	140	1/1·3
XXVIII	6	49	78	62	157	116	1/1·4
XXIX	4	5	94	55	123	92	1/1·3

with Sturmer Pippin, because there is no reason to believe that acetaldehyde cyanhydrin would yield free acetaldehyde during the operations of quantitative estimation, and because the results described in the subsection above prove that free acetaldehyde accumulates in notable concentrations. The hypothesis we put forward is that in HCN zymasis, as in CO_2 zymasis, the considerable accumulation of acetaldehyde results from the partial inhibition of those oxido-reductases in the zymase complex which are concerned with the reduction of acetaldehyde.

Now although CO_2 zymasis is induced by the inhibition by carbon dioxide of enzymes that take part in aerobic oxidations, high acetaldehyde accumulation in

[1] We have already stated (p. 220) that these numbers may err on the high side, but not so much as to invalidate our general conclusions.

this form of zymasis necessarily depends upon the presence of oxygen in the atmosphere around apples (Thomas, 1925). This effect of oxygen in gas mixtures of carbon dioxide and oxygen is reduced as the external concentration of oxygen is lowered to a value less than that (say 3 %) which would extinguish anaerobic respiration in apples placed in a gas mixture of nitrogen and oxygen. It is possible that certain aerobic dehydrases that are not poisoned by carbon dioxide may, in the presence of adequate oxygen, depress the activity of aldehyde oxido-reductase.

We have no convincing evidence that oxygen plays a similar role in modifying HCN zymasis. It is clear that the inhibition of aldehyde oxido-reductase cannot be ascribed to oxygen, acting independently of HCN, because acetaldehyde accumulation is augmented when dosing with cyanide is increased at constant external oxygen concentration. Further, we have obtained evidence (§ 8) that HCN differs from CO_2 in that it partially arrests the reduction of acetaldehyde to ethyl alcohol under anaerobic conditions. Although poisoning by cyanide may be the main cause of the inhibition, aerobically as well as anaerobically, of oxido-reductase, it is conceivable that there may also be an oxygen effect under aerobic conditions. This would explain differences found in parallel experiments on Newton Wonder, in which the D/Z ratio was about 1/2·5 in aerobic HCN zymasis, and varied between 1/5 and 1/10 in anaerobic zymasis in the presence of cyanide; but it is admitted that other explanations can be given.

5. HCN ZYMASIS AS DISTINCT FROM INJURY ZYMASIS AND SENESCENCE ZYMASIS

The progress of HCN *zymasis in uninjured tissue*

Sufficient evidence that HCN zymasis is not caused by and does not necessarily lead to the disorganization of protoplasm, is given by the results of XI, XII and XIII, on Dunn's Favourite (Fig. 1), in which zymasis progressed for a fortnight before any injury was apparent, and of I and VI–X on Newton Wonder (Tables 1 and 2) dosed with weak cyanide, in which there was some zymasis but no injury. Stronger cyanide treatment led to severe injuries after a few days in Newton Wonder (Table 1), Granny Smith (Table 3), Sturmer Pippin (Fig. 2), and other varieties, but in all these experiments vigorous HCN zymasis had preceded tissue browning. For example, in III the cyanide number had reached 7 after 3 days, and the alcohol number was then 91, to which the acetaldehyde number contributed 32 units. It was not until the fourth day that injury was observed.

Behaviour upon returning to pure air apples containing cyanide and products of zymasis

An argument that has to be met is that cyanide induces zymasis by bringing about, prematurely, autolysis of the type which occurs during senescence (Fidler, 1933*a*). If it is allowed that those physico-chemical changes in cells which lead to autolysis are irreversible, the possibility that HCN zymasis and senescence zymasis are identical may be at once ruled out, because we have shown in some of our experiments that zymasis slows down, and, indeed, soon stops, when apples

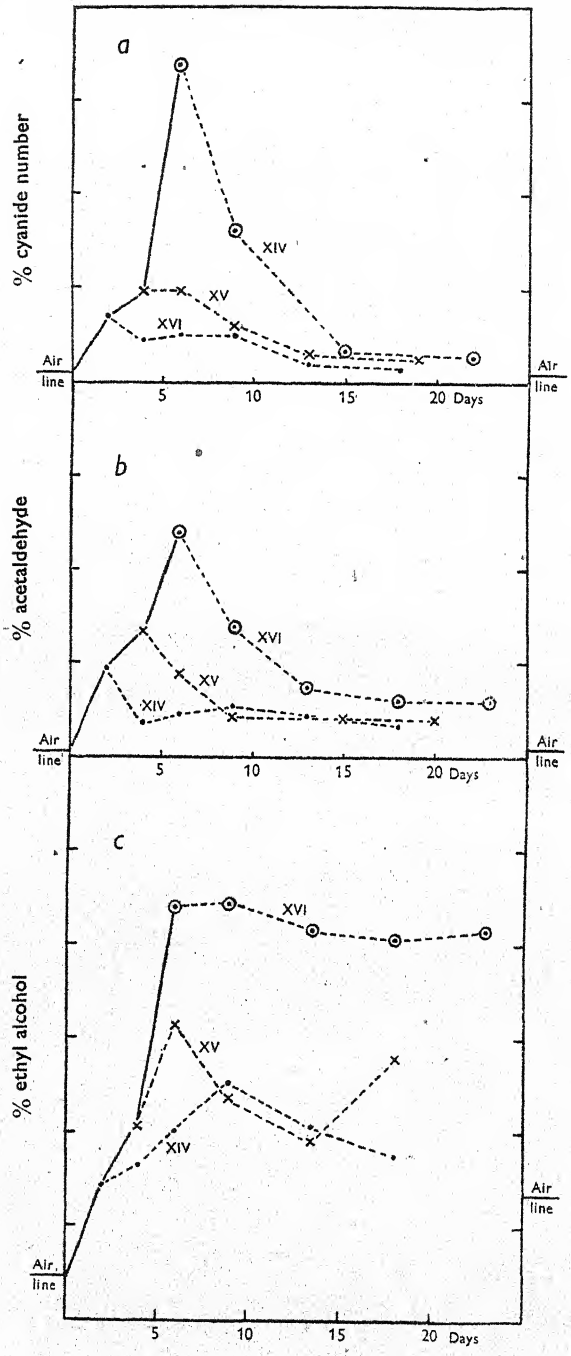

Fig. 3 *a*, *b* and *c*. The continuous lines show the changes in the cyanide number and in the ethyl alcohol and acetaldehyde numbers of Dunn's Favourite (XIV, XV, XVI), exposed for 2 days (•), 4 days (×), and 6 days (⊙), at room temperature in May and June 1932, to air that had previously been passed through 5 % KCN. The interrupted lines show subsequent changes when the apples were transferred to pure air.

are removed from air containing HCN vapour to pure air. The charted records in Fig. 3 *a*, *b* and *c*, show the progress over a period of 6 days of cyanide accumulation and of zymasis by Dunn's Favourite when under cyanide treatment. They also show the changes in the concentrations of the accumulated cyanide, ethyl alcohol, and acetaldehyde, when samples of fruit were replaced in pure air after 2 days (XIV), 4 days (XV), and 6 days (XVI). It will be observed that even after 2 days of cyanide treatment the alcohol number was higher than the *final* alcohol number of apples used in the control experiments in pure air. There is no doubt that cyanide accumulation had led to an acceleration of the rate of zymasis. We infer that this was not a result of accelerated senescence, since, when the cyanide treatment was stopped, the regularly progressing increase of the alcohol number was arrested. Zymasis at a diminished rate may have continued for a few days under the influence of the residual cyanide in the tissue; but the experimental results, especially those for XVI, suggest that this did not last long. Newton Wonder under dilute cyanide behaved like Dunn's Favourite. In general the values of the alcohol numbers (y) below those printed in italics in Table 2 suggest that, after a day or two in pure air, subsequent to cyanide treatment, zymasis was no longer occurring in V–X. We conclude therefore that HCN zymasis is distinct from senescence zymasis as well as from injury zymasis, and hold to the explanation given on p. 226 that it results from the specific action of cyanide upon oxidation systems in living apple tissue.

6. The reversibility of the inhibition by cyanide of oxidative metabolism in uninjured tissue

The fact that zymasis soon stopped in XIV–XVI on Dunn's Favourite, and V–X on Newton Wonder, when apples were transferred to pure air from an atmosphere containing HCN vapour, probably means that, in pure air, those inhibitory processes which had previously led to zymasis were quickly reversed, and normal powers were restored to cells of consuming zymase cleavage products in aerobic metabolism. Supporting evidence for this view has come from the finding of James & Hora (1940) that the oxygen uptake of barley leaves is reversibly inhibited by $M/500$ HCN. We have not yet obtained such clear evidence of reversibility from our measurements of gaseous exchanges in the apple. Indeed, it must be admitted that we cannot yet cite evidence of a reduction in oxygen uptake that is accompanied by zymasis, and which is reversed, with the stoppage of zymasis, when cyanide is removed from the experimental system (see also, p. 251).

It is interesting to note that there was a tendency in XIV–XVI and V–X for hydrogen cyanide and acetaldehyde to disappear from fruit replaced in air. The accumulated ethyl alcohol remained in the tissue. It did not escape to any appreciable extent as vapour, nor was it converted by metabolism or by purely chemical reactions into other products. The possible fates of HCN and of acetaldehyde in cells living aerobically are still under consideration.

In II–V on Newton Wonder (Table 1) and XXII on Granny Smith (Table 3) the cyanide concentration diminished very little in apples that were placed in air

after strong cyanide treatment. The concentrations of ethyl alcohol and acetaldehyde increased, possibly as a result of injury zymasis as well as of continued HCN zymasis. The inhibition of processes of aerobic metabolism had persisted. Indeed, it became more acute. Our measurements of gaseous exchanges support this conclusion. We note that James & Hora (1940) found that the oxygen uptake of barley leaves was completely inhibited by $M/50$ HCN, and that the inhibition was not reversed by the removal of the cyanide supply.

7. ALDEHYDE POISONING AS A CAUSE OF THE OBSERVED INJURIES DURING HCN ZYMASIS

Prussic acid is a plant poison to which apples show a measure of tolerance. Rough calculations from cyanide numbers indicate that internal concentrations of HCN less than $M/500$ may be endured for considerable periods, but that concentrations of $M/250$ may be lethal in a few days. It is especially suggested that apples containing cyanide at concentrations insufficient to damage the fruit may be poisoned by the acetaldehyde that accumulates in them as a result of HCN zymasis. Injuries to Newton Wonder (Table 1) resembled those which this variety showed when suffering from aldehyde poisoning after CO_2 zymasis and when half apples had been exposed to the vapour of acetaldehyde (Thomas, 1929). Moreover, the aldehyde number (d) of injured apples in these experiments was over 30, a value that is higher than the minimum value, viz. 10, found for apples suffering from aldehyde poisoning after CO_2 zymasis. In Granny Smith (Table 3) the d value at injury was over 20. For injured Sturmer Pippin (Table 5) d values ranged from 24 to 62; in XXIX the cyanide number was as low as in the experiments on Newton Wonder, but, in the others, so high that the flesh tissue may have been directly poisoned by HCN. The resistance of Dunn's Favourite (Fig. 1) provides further supporting evidence. In XIII cyanide accumulated in this variety to a value of 5 in a fortnight, and ethyl alcohol to 150; but the aldehyde number did not reach 12, and during this period the fruit was uninjured.

8. THE INFLUENCE OF HYDROGEN CYANIDE ON ANAEROBIC ZYMASIS

The rates of anaerobic zymasis in the absence (Z_{N_2}) and in the presence (Z_{N_2+HCN}) of cyanide

It is probable that zymasis proceeds at its maximum possible rate in the complete absence of oxygen and of poisonous substances such as hydrogen cyanide. For the apple in pure nitrogen at room temperature (say 15° C.) rates of Z_{N_2} greater than 50 have frequently been obtained. The maximum rates of *aerobic* HCN zymasis ($Z_{Air+HCN}$) have usually been very much lower than the lowest values ever found for Z_{N_2}, possibly because, in some experiments, oxidation systems were incompletely inhibited, or because, in others, in which inhibition was complete, zymase activity had been depressed owing to the presence of cyanide in the living cells.

That strong cyanide may depress the activity of zymase acting *anaerobically* in apple cells is indicated by the results that are graphically represented in Fig. 4.

In XVIII the average Z_{N_2} value over the whole period was just under 70. During the first day in XIX, while the cyanide number (y) gradually increased to 2·5, Z_{N_2+HCN} was about 55. This rate probably decreased as the y number increased and the exposure of cells to cyanide was prolonged. Certainly in subsequent days

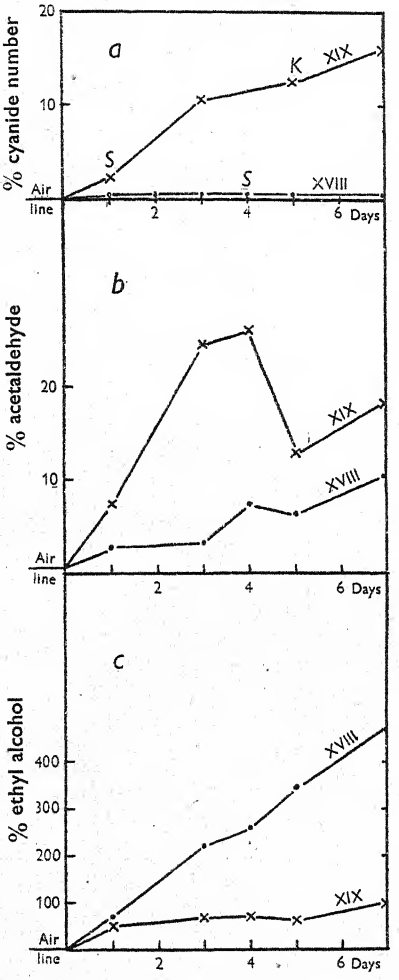

Fig. 4.

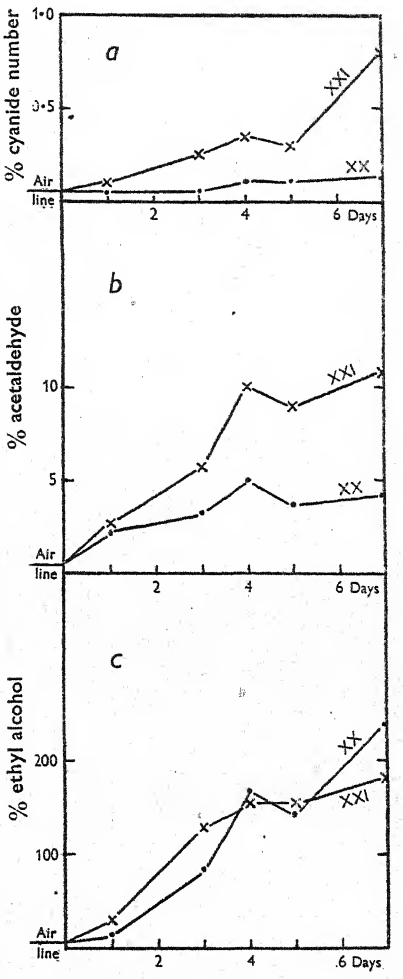

Fig. 5.

Figs. 4, 5. Changes in the cyanide number and in the acetaldehyde and ethyl alcohol numbers of Newton Wonder kept at room temperature in February and March 1932, in pure nitrogen (•) and in nitrogen containing the vapour of HCN (×). In XVIII and XIX cyanide treatment was relatively strong (Fig. 4), and in XX and XXI relatively weak (Fig. 5). The first sign of injury is indicated by *S*, and extensive damage by *K*.

the rate was strongly retarded, and with a y number of 10 on day 4 it had become zero. Since the fruit was injured after one day and severely damaged after three days the general disorganization of the protoplasm, rather than the direct poisoning of zymase by cyanide, may have been the cause of the inhibition. It is an old

doctrine that, although zymase may be extracted from killed cells, it shows activity in tissues only while the cells are still living. An experiment (XXIV) on Newtown Pippin at 12° C. gave similar results (Table 6). During the opening 8 days Z_{N_2} was 26, while Z_{N_2+HCN} was only 14 in the parallel experiment in which the cyanide number reached the y value of 2·2. During the subsequent 14 days injuries of increasing severity were observed, and Z_{N_2} averaged 17·5, while Z_{N_2+HCN} was only 5, with a final y number of 14·4.

The anaerobic behaviour of apple cells in the presence of cyanide resembles that of barley leaves (James & Hora, 1940) in that depressed metabolism results from prolonged exposure to cyanide, whereas resistance is shown in short exposures, especially when the cyanide concentration is low. For example, we may infer that zymase in apple cells tolerated cyanide during the first half of XXI (Fig. 5), since, while the cyanide number increased to 0·25, the value of Z_{N_2+HCN}, averaging about 35, remained steady and was not appreciably different from that found for Z_{N_2} in the parallel experiment XX. But during the second half the absorbed cyanide, with a final y number of 0·8, had caused a three-fold decrease in the rate of zymase cleavage. In XX, Z_{N_2} was still averaging 35, but in XXI the average value of Z_{N_2+HCN} had fallen to 10.

Comparison of $Z_{Air+HCN}$ *and* Z_{N_2+HCN}

With cyanide concentrations that do not retard the rate of zymase cleavage, i.e. when the rates Z_{N_2} and Z_{N_2+HCN} are equal, the quotient $100Z_{Air+HCN}/Z_{N_2}$ would give a measure of the percentage inhibition by cyanide of oxidative metabolism beyond the level associated with an external oxygen concentration of 3 %.[1] We cannot illustrate this point yet, because we have not obtained evidence so far of aerobic HCN zymasis with cyanide numbers of the order 0·25, i.e. of the order to which zymase was indifferent in the anaerobic experiment XXI.

In most of our *aerobic* experiments such concentrations were developed and maintained in tissues as would have depressed anaerobic zymasis, i.e. Z_{N_2+HCN} must have been less than Z_{N_2}. The quotient $Z_{Air+HCN}/Z_{N_2+HCN}$ can be evaluated from the results of II (Table 1) and XIX. The temperature and the rate of cyanide accumulation were approximately the same during the opening phases of these experiments. From II we get a value of about 35 for $Z_{Air+HCN}$, and from XIX a value of 55 for Z_{N_2+HCN}. We infer that in the aerobic experiment the cyanide had caused a 65 % reduction in the oxidative metabolism of zymase cleavage products beyond the level of activity that would have prevailed in apples surrounded by 97 % nitrogen

[1] Results obtained in the investigation of the influence of oxygen on zymasic metabolism (Thomas & Fidler, 1933) illustrate the use of this measure. In parallel experiments on Newton Wonder at 23° C. the average rate of anaerobic zymasis (Z_{N_2}) was 110, and the rates of low-oxygen zymasis ($Z_{low\ O_2}$) were 72 with 1 % oxygen, 36 with 2 %, and zero with 3 %. Substituting these values in the quotient $100Z_{low\ O_2}/Z_{N_2}$, we conclude that a fall in the oxygen concentration from 3 to 2 % reduced oxidative activity by more than 30 %, and from 3 to 1 % by more than 65 %. We emphasize the point that 110 was the maximum possible rate of zymasis at 23° C., as it was found when aerobic processes were completely suppressed and zymase activity was not impaired by the presence of a poison. We also recall that oxidative activity was reduced without accompanying zymasis when the oxygen concentration was lowered from 20 % to some value just below 3 %.

plus 3 % oxygen. At a later stage in these two experiments, as the apples were progressively injured, the declining rates $Z_{Air+HCN}$ and Z_{N_2+HCN} became about the same. We infer that at this stage the activities of oxidation enzymes concerned with the aerobic metabolism of products of zymase cleavage were completely inhibited.

D/Z ratios

The graphic records in Figs. 4 and 5 show that acetaldehyde accumulated under anaerobic conditions more rapidly and to a greater amount when cyanide was present. As a result of strong cyanide treatment the D/Z ratio changed from 1/50 to 1/5 (XVIII and XIX), and with the weaker doses (XX and XXI) the change was from 1/60 to 1/20. In XXIV on Newtown Pippin (Table 6) during the first 8 days the increase of 2·2 in the cyanide number caused not only a reduction in the rate of zymasis (p. 253) but also a substantial decrease in the D/Z ratio. During the

Table 6. *The influence of* HCN *on the accumulation of acetaldehyde in the anaerobic zymasis of Newtown Pippin* (*XXIV*) *at* 12° C.

Gas	Consecutive periods days	Increase in cyanide number	Increase in alcohol number	Increase in acetaldehyde	D/Z
Pure N_2	8	0·3	206	3	1/69
	14	0·1	246	< 1	< 1/246
N_2+HCN vapour	8	2·2	110	9	1/12
	14	12·2	72	29	1/2·5

The progress of anaerobic respiration in this experiment is illustrated in Fig. 8 on p. 253.

second phase of 14 days there was practically no further accumulation of acetaldehyde in pure nitrogen (cf. Thomas, 1925), but the values for acetaldehyde and for the D/Z ratio show that with an increase during this period of 12·2 in the cyanide number, there was considerable arrest at the acetaldehyde stage of such zymasis as continued in the dying apple cells.

The evidence, as we have already pointed out (p. 230), suggests that cyanide may bring about the differential depression of the activity of that part of the zymase system which is concerned with the reduction of acetaldehyde to ethyl alcohol.

Summary

1. Placed in air containing the vapour of hydrogen cyanide, several varieties of apple have been shown to absorb this poison. Their cyanide numbers (y) progressively increased. Up to a certain value the external concentration of cyanide determined the rate of cyanide accumulation (Y). Increase above this value did not enhance the rate unless the fruit was injured. Presumably in healthy apples structural factors exerted control over the rates of HCN uptake and accumulation.

2. When Y exceeded a certain value, ethyl alcohol and acetaldehyde accumulated in the flesh tissue, i.e. HCN zymasis was induced. The sum of the ethyl alcohol concentration (e) and the concentration of acetaldehyde (d) gives what we call the alcohol number (z). In the experiments described in this paper the rate of HCN zymasis (Z), evaluated from the measured daily increments in z, was limited by Y.

3. During zymasis in the presence of cyanide, under anaerobic as well as aerobic conditions, the reduction of acetaldehyde to ethyl alcohol was retarded. The extent of the retardation was determined by Y, and was possibly augmented in the presence of oxygen. Certainly, in some of the experiments on aerobic HCN zymasis, the rate of accumulation of acetaldehyde (D) and the maximum concentrations of acetaldehyde that were developed were strikingly high, and more akin to those found during CO_2 zymasis, i.e. zymasis induced by high concentrations of CO_2 in the presence of abundant oxygen, than during anaerobic zymasis in pure nitrogen or pure CO_2. The values of e were correspondingly lower than after anaerobic zymasis. D/Z ratios have been evaluated to determine the fraction that accumulated of the total acetaldehyde produced in zymasis.

4. Apples were soon injured when treated with relatively high concentrations of cyanide. Evidence was obtained that poisonous products of zymasis, particularly acetaldehyde, aggravated the toxic conditions then prevalent.

5. Apples when treated with relatively weak doses of cyanide showed zymasis and survived uninjured, both when under treatment and after they were replaced in pure air. Moreover, in strongly dosed apples, HCN zymasis proceeded for two or more days before injury was seen. The conclusion drawn is that HCN zymasis is distinct from injury zymasis and senescence zymasis.

6. If we accept certain well-known current theories of the mechanism of the aerobic respiration of hexose sugar and of alcoholic fermentation (see Thomas, 1940), the induction of all the forms of zymasis so far studied, and the peculiarities of these forms, can be explained in terms of the differential inhibition of the enzymes that participate in the processes of respiration and fermentation.

The induction of HCN zymasis is explained if we assume that the activities of one or more of the oxidation enzymes that act in concert with zymase in the normal aerobic respiration of hexose sugar, are poisoned by HCN, CO_2 in high concentration, and H_2S; weakened during senescence and when tissues are injured; depressed by lowering the oxygen concentration; or completely inhibited under anaerobic conditions; while zymase activity is increased, unaffected, or reduced by a smaller amount than is the activity of the most sensitive of the executive oxidation enzymes.

Differences in the values of D/Z ratio found for different forms of zymasis are explained if we assume that the activities of oxido-reductases in zymase are depressed to a greater extent than is the activity of the other components of the zymase complex (a) by HCN and H_2S, and (b) by aerobic dehydrases that are insensitive to HCN, H_2S and CO_2, when these dehydrases are operating with higher external concentrations of molecular oxygen than those which extinguish anaerobic metabolism, i.e. about 3 % for the apple.

7. The cessation of zymasis when apples containing relatively low concentrations of cyanide were returned to air, suggests that the inhibition of oxidative metabolism by cyanide may be reversible. The concentrations of HCN and acetaldehyde gradually diminished, but the accumulated ethyl alcohol remained in the tissue.

8. The relative values of the rates of aerobic HCN zymasis ($Z_{\text{Air+HCN}}$) and of anaerobic zymasis ($Z_{\text{N}_2\text{+HCN}}$) in apples receiving similar cyanide treatment have been used for measuring the degree to which the presence of HCN in cells, living aerobically, inhibited the oxidative metabolism of zymase cleavage products. Evidence of complete inhibition in air was only obtained for injured fruit.

Acknowledgements and references will be found at the end of the next paper (pp. 260, 261).

STUDIES IN ZYMASIS

IX. THE INFLUENCE OF HCN ON THE RESPIRATION OF APPLES, AND SOME EVALUATIONS OF THE 'PASTEUR EFFECT'

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(With 8 figures in the text)

Introduction

For many years special attention has been paid at Newcastle to the measurement of the products of the catabolism of carbohydrates under conditions in which anaerobic glycolysis, taking the form of zymasis, proceeds collaterally with aerobic respiration. In some respects the experimental investigations have been influenced by the famous researches of Meyerhof (1925) on the metabolism of yeast, of Warburg (1926) on the metabolism of healthy and cancerous animal tissues, and of Blackman (1928) on the respiration of the apple.

In an earlier paper (Thomas & Fidler, 1933) we maintained that theoretical analysis is facilitated if measurements are made of changes in the value of the alcohol number as well as of the CO_2 output, when apples are respiring under anaerobic conditions or in gas mixtures containing subnormal concentrations of oxygen. In the work described in the present paper we have widened the scope for such analysis, because in some of our experiments on aerobic HCN zymasis, we have measured the consumption of oxygen as well as the CO_2 output and the changes that occurred in the alcohol number. As far as we know these experiments were the first to be performed on the higher plants in which these three magnitudes were simultaneously determined. They represent a stage in the forward development leading, it may be hoped, to the ideal investigation, in which, in addition, measurements are made of changes in the concentrations of such sugars or other substrates as are consumed in anaerobic glycolysis as well as in aerobic respiration.

Our chief tasks in the present paper are to consider whether our measurements provide evidence (i) for attributing to a depression in aerobic respiration the induction and continued progress of aerobic HCN zymasis, which has been described in the preceding paper, and (ii) for demonstrating the operation of a 'Pasteur effect' in apples, and, if it is proved to operate, of measuring its magnitude.

1. Experimental methods and the expression of results

All the experiments except XXIII and XXIV on Newtown Pippin were carried out at a constant temperature of 23° C. in the Botany Department, Newcastle-upon-Tyne, during the period 1929–33. Some of them ran parallel with those

described in the preceding paper. XXIII and XXIV were performed at 12° C. in the Low Temperature Research Station, Cambridge, in July 1936. We thank Dr Franklin Kidd for the facilities given.

In most of the experiments apples were placed for a period in air containing the vapour of HCN, until evidence was obtained that HCN zymasis was in progress. For the determination of the respiration or of the respiratory quotient (R.Q.), the apples were returned to pure air. Changes in the cyanide number and alcohol number were measured from time to time by methods already described (p. 220), In measuring respiration we used an air stream, any HCN given off by the fruit being removed by absorption in silver nitrate dissolved in dilute nitric acid. The only difference in conducting the control experiments was that the fruit was kept continuously in pure air. CO_2 output was measured in the usual way by determining the amount of CO_2 absorbed in unit time by a solution of baryta. In measuring the R.Q. we transferred samples from the poisoning chambers to pure air in closed desiccators, and we used the Haldane gas-analysis apparatus to determine such changes in gas composition as occurred over a period.

In XXIII on Newtown Pippin another series of values for CO_2 output was obtained by analysing, every day, with the Haldane apparatus, samples of gas issuing from the respiratory chambers. The values so obtained agreed closely with those found by the baryta method. Oxygen uptake was simultaneously measured. Inasmuch as cyanide treatment was continuous in this experiment, in calculating the CO_2 output, we have made corrections for the presence of HCN in the air passing over the fruit.

In XXII on Granny Smith the CO_2 output and oxygen uptake of the cyanide-treated apples and of the controls were measured in the apparatus described by Barker (1931). In our experiment, however, the apples were placed in the respiratory chamber after they had been dosed with HCN, and the HCN generator was not included in the circuit.

We have charted our experimental results in Figs. 1–8; and such secondary data as have been calculated are expressed in Tables 1–5 as mg. or c.c. HCN, CO_2, oxygen, alcohol number, carbon unit or hexose equivalent, per 100 g. fresh-weight of tissue. Some of the discussions are based on such differences in the values found for the total amounts of production or consumption over a period as are attributable to varying the concentrations of HCN; others on apparent changes in the average rates of the process during the course of the period.

Thanks to the spacious accommodation available for carrying out experiments at constant temperature at Cambridge, the samples used in XXIII and XXIV on Newtown Pippin were sufficiently large to be truly comparable. In all the Newcastle experiments, which were performed on several varieties of apple (see p. 219), the samples were small; indeed, measurements were often made of the respiration and zymasis of single apples. Clearly, individual variation within a sample would be an important contributory cause of such differences as were found in the magnitudes measured in these experiments. Accordingly, most of the conclusions drawn have been based on similar drifts of values that have been encountered in

several experiments made under comparable conditions either on the same or on different varieties of apple.

It will be convenient to represent certain of the magnitudes measured by experiment, or calculated as secondary data, by the symbols defined below, which are based on those used by Blackman (1928). Additions might be made to the list (e.g. symbols for the hexose equivalents in mg. or mg.mol. of the various fractions of CO_2 output), but, as it stands, it is a sufficient witness of the extension of knowledge to be gained by measuring organic products of zymasis as well as CO_2 output when anaerobic glycolysis contributes to the total catabolism of carbohydrates.

CO_2 output in air

(1) $CO_2.O.R._{Air}$ — CO_2 output in normal aerobic respiration in air. This is measured directly. It is convenient in some of the tables to describe this magnitude for the controls as $CO_2.T.$

CO_2 output in air containing HCN *vapour*

(2) $CO_2.T._{Air+HCN}$ — Total CO_2 output in catabolic processes. This is directly measured.

(3) $CO_2.Z._{Air+HCN}$ — CO_2 output from zymasis. This is calculated from measured changes in the alcohol number.

(4) $CO_2.O.R._{Air+HCN}$ — CO_2 output in such normal aerobic respiration as persists. This is calculated from measurements of oxygen uptake.

(5) $CO_2.Res._{Air+HCN}$ — Residual CO_2 output not accounted for by (3) and (4), i.e. it is $[CO_2.T._{Air+HCN} - (CO_2.Z._{Air+HCN} + CO_2.O.R._{Air+HCN})]$. Clearly, when $CO_2.Res._{Air+HCN}$ is zero, $CO_2.O.R._{Air+HCN}$ is given, without measuring oxygen uptake, by

$$(CO_2.T._{Air+HCN} - CO_2.Z._{Air+HCN}).$$

CO_2 output under anaerobic conditions in pure nitrogen

(6) $CO_2.N.R._{N_2}$ — Total CO_2 output in catabolic processes. This is directly measured.

(7) $CO_2.Z._{N_2}$ — CO_2 output from anaerobic zymasis, and is unquestionably of metabolic origin. This is calculated from changes in the alcohol number.

(8) $CO_2.Res._{N_2}$ — Residual anaerobic CO_2 output, which is of unknown origin. It is given by $(CO_2.N.R._{N_2} - CO_2.Z._{N_2})$.

CO_2 output under anaerobic conditions in nitrogen containing HCN *vapour*

(9) $CO_2.N.R._{N_2+HCN}$ — See (6).

(10) $CO_2.Z._{N_2+HCN}$ — See (7).

(11) $CO_2.Res._{N_2+HCN}$ — See (8).

The suffixes will be omitted when the context makes the meaning clear.

In thus analysing our results in the present paper we are simply developing a method which we first used some years ago (Thomas & Fidler, 1933) when we

resolved $CO_2.T._{low\,O_2}$ into $CO_2.O.R._{low\,O_2}$ and $CO_2.Z._{low\,O_2}$. We recall that in ageing and in injured apples, and usually to a slight extent in healthy 'conditions controls' living in air, the $CO_2.T._{Air}$ is compounded of $CO_2.Z._{Air}$ as well as $CO_2.O.R._{Air}$.

2. The influence of cyanide on the rates of oxygen uptake and aerobic CO_2 output

Preliminary experiments

It was expected that in apples treated with HCN there would be a depression in the rates of oxygen uptake and of aerobic CO_2 output before zymasis was set in motion, as happens when the oxygen concentration is reduced in the atmosphere around respiring apples (p. 226); but no instance of such behaviour was revealed

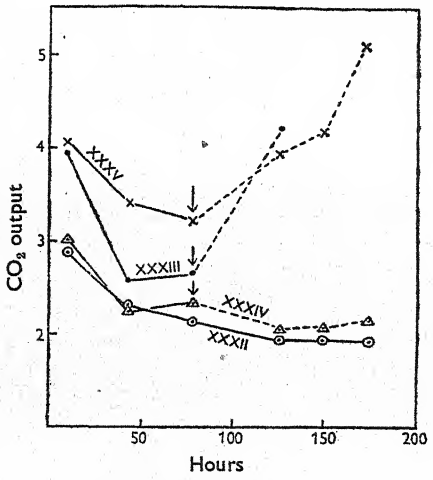

Fig. 1. The influence of weak HCN on the CO_2 output in mg./hr./100 g. fresh-weight tissue at 23° C. of Newton Wonder (March, 1932). XXXII was the air control. The arrow indicates the time at which the apples were first treated with HCN in XXXIII, XXXIV and XXXV.

	XXXII	XXXIII	XXXIV	XXXV
Final cyanide number	0·2	0·6	0·5	0·9
Final alcohol number	21	70·7	43	160

in our preliminary experiments. In most of these, HCN zymasis was quickly induced in the treated fruit, and the rate of CO_2 output was usually enhanced. For example, the rate increased soon after dosing in XXXIII–XXXV on Newton Wonder (Fig. 1), in XXXVII and XXXVIII on Dunn's Favourite (Fig. 2), and possibly in XXII on Granny Smith (Fig. 3). At the end of all these experiments the alcohol numbers were much higher in apples that had absorbed HCN than in untreated apples. In the latter, senescence zymasis took place to a varying extent. The results of XXXII–XXXV provide evidence that augmented CO_2 output may be related to the intensity of zymasis, which, as we have seen (p. 226), is determined by the rate of cyanide accumulation.

The first experimental demonstration that a reduction in the rate of oxygen uptake may be an accompanying feature of HCN zymasis came from XXII on Granny Smith (Fig. 3). Since the results of this experiment did not inform us whether oxygen uptake was reduced before HCN zymasis was brought into operation, we still lacked solid evidence for inferring that the zymasic process was occasioned by the inhibition of oxidative activities.

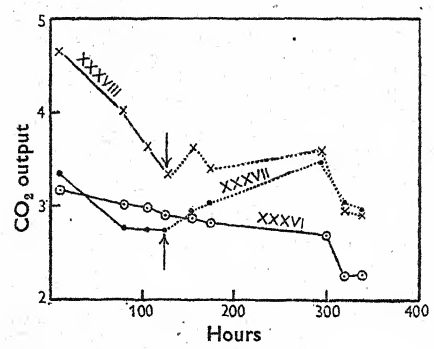

Fig. 2. The influence of HCN of moderate strength on the CO_2 output in mg./hr./100 g. fresh-weight tissue of Dunn's Favourite at 23° C. XXXVI was the air control. The arrows indicate the time at which the apples were first treated with HCN in XXXVII and XXXVIII.

	XXXVI	XXXVII	XXXVIII
Final cyanide number	0·2	3·1	6·8
Final alcohol number	90·2	177	201

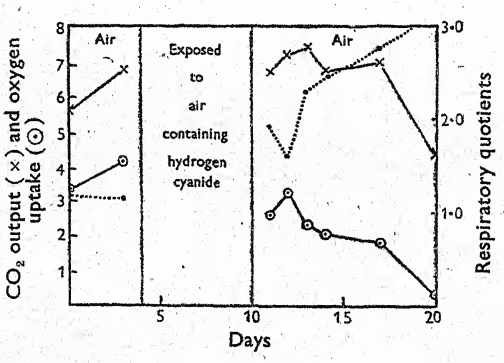

Fig. 3. The influence of HCN of moderate strength (*a*) on the CO_2 output (×) and oxygen uptake (⊙) of Granny Smith at 23° C., expressed as mg./hr./100 g. fresh-weight tissue, and (*b*) on the R.Q. Data of increases in the cyanide number and alcohol number in this experiment (XXII) are recorded in Table 3 (p. 224).

In all out prolonged experiments the CO_2 output fell off to zero after HCN treatment had injured the apples. In XXII, when Granny Smith showed injury, retardations in oxygen uptake became stronger in spite of the fact that in the poisoned tissue, oxygen was absorbed in oxidase brownings in addition to being used in such aerobic metabolism as persisted. Before the end of the experiment, oxygen

uptake had practically ceased, although the apples were not completely brown, while CO_2 was still being produced, presumably for the most part by zymasis.

Some varieties were soon injured under conditions that did not for some time cause visible damage to others. Thus rather weak cyanide treatment reduced, in 2 days, the CO_2 output of Beauty of Bath to one-third of its air value, and the flesh tissue was injured. In the same month (August 1930) Australian Cleo withstood these conditions for 5 days, showing a rise in the hourly rate of CO_2 output from 3 to 3·5 mg. % fresh-weight tissue. There was a fall to 1·4 mg. after further exposure for 4 days to the same conditions.

We have found that the production of CO_2 by apple tissue may be quickly stopped by exposing slices to air containing strong HCN. The general poisoning involves zymase as well as oxidation enzymes, and death may take place without oxidase browning.

Although all the possibilities of variation in behaviour had not been exhausted in these preliminary experiments, which were carried out concurrently with some of those described in the preceding paper, it had become evident that the problem of ascertaining the respiratory responses of apples to cyanide treatment would not be easily solved, and that the further elucidation of the problem would require the performance of a series of experiments, each one of which would demand whole-time attention for a considerable period.

The first of these experiments was performed by Dr Fidler at Cambridge, and will be described below. The principal facts established at Newcastle formed the basis of this experiment. These were that when the HCN concentration in an apple reaches a value that induces vigorous HCN zymasis in the flesh tissue without poisoning zymase, the rate of oxygen uptake tends to fall, and the rates of CO_2 output and hexose catabolism tend to rise. It was hoped that XXIII on Newtown Pippin would provide evidence of what occurs before HCN zymasis begins to operate, in addition to providing more accurate data for determining the magnitude and significance of such changes as might be brought about by the action of HCN.

Oxygen uptake and CO_2 *output by Newtown Pippin* (XXIII)

In Fidler's experiment the respiratory behaviour of samples of Newtown Pippin consisting of twelve apples each was studied at 12° C. The sample to be treated with cyanide was placed for a preliminary period of about 5 days in pure air. It absorbed oxygen and gave off CO_2 at rates not very different from those shown by the control sample, which was kept continuously in pure air (Figs. 4, 5). For this and for other reasons (p. 251) the two samples may be regarded as having been comparable.

The rate of respiration of the control sample remained steady throughout the experimental period of 36 days, excepting for a possible tendency to rise towards the end of the experiment. This tendency is more clearly illustrated in Fig. 7, in which the results are, for other purposes, expressed in a different way.

The object of treatment with dilute doses of cyanide was to bring about sustained HCN zymasis after a preliminary phase of reduced aerobic respiration without zymasis. It was intended subsequently to study the respiratory behaviour

of the treated apples after they were returned to pure air, in order to determine whether any of the changes induced by cyanide are reversible. We did not fully succeed in our objects, because during the first phase of the cyanide treatment, which lasted 9 days, viz. from day 5 to day 14, although the cyanide number increased to 1·2 mg. %, the increase by 10 mg. % in the alcohol number provided only doubtful evidence that any HCN zymasis had occurred at all. Accordingly, Dr Fidler decided to increase the strength of cyanide dosing during the second phase of the experiment, which lasted for 15 days, viz. from day 15 to day 29. The important positive results obtained during this phase will be analysed in later

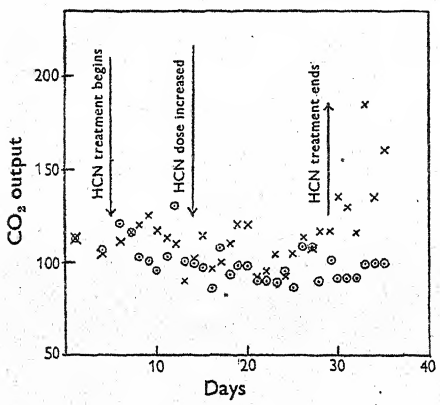

Fig. 4.

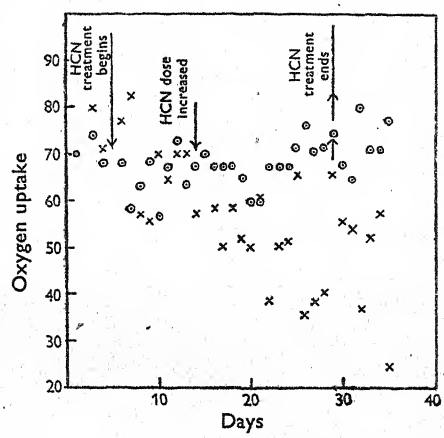

Fig. 5.

Figs. 4, 5. The influence of HCN on the rates of CO_2 output (Fig. 4) and oxygen uptake (Fig. 5) by samples of Newtown Pippin (XXIII) treated with HCN (×) and of a control sample (⊙) in mg. 10 kg. fresh weight per hour at 12° C. For description, see text. Cyanide numbers and increases in alcohol numbers attributable to the action of HCN are given below.

Days after cyanide treatment began	Cyanide number	Alcohol number
9	1·2	10
24	8·6	147
30	4·7	362

sections of this paper. In the 15 days the cyanide number increased to 8·6 and HCN zymasis was a vigorous process, seeing that at the end the alcohol number was 147. As it was not feasible to determine from day to day the progress of alcohol accumulation, we do not know exactly when HCN zymasis began, but the definite and sustained rise in the R.Q. (Fig. 7) after day 15 strongly suggests that zymasis became an active process shortly after the cyanide dose was increased, and that during the course of the second phase it grew in vigour.

For the 6 days from day 30 to the end of the experiment the treated apples, some of which were already injured, respired in pure air. During this period the cyanide number fell to 4·7. Injuries became increasingly severe, and all but two of the twelve apples were completely brown at the end of the experiment. The

increase of the alcohol number to 362 may, therefore, have resulted from injury zymasis as well as from such HCN zymasis as continued under the influence of the residual cyanide in the fruit.

The results as plotted in Figs. 4 and 5, which form the basis for the calculations of the secondary data discussed later in this paper, do not show clearly any changes in respiration brought about by cyanide treatment until after the external HCN concentration had been increased. Although widely scattered, the values for oxygen uptake whilst zymasis was a vigorous process unmistakably drift downwards until the end of the experiment, in spite of the fact that injured apples were absorbing the oxygen used in the production of brown products, and the fact that in the control experiments the oxygen uptake tended to increase. As in XXII on Granny Smith, the operation of HCN zymasis in XXIII was associated with a reduction in the rate of oxygen uptake; but the results as set out in Fig. 5 do not clearly disclose the critical fact that oxygen uptake may have been depressed before HCN zymasis was induced.

Two conclusions may be drawn from the values obtained for the CO_2 output by the treated fruit (Fig. 4); first, that the increased cyanide dosing, which led to the occurrence of HCN zymasis, did not cause a reduction in the rate of CO_2 output; and, secondly, that CO_2 output showed a definite tendency to increase during the last 10 days of the experiment. The behaviour of Newtown Pippin during this phase clearly resembled that of some of the varieties used in our preliminary experiments.

There still remains the difficult problem of ascertaining whether our results reveal changes brought about by HCN in the rates of gaseous exchanges before the induction of HCN zymasis. The problem might have been simpler had respiration settled down to a regular rate immediately the apples were placed in the respiratory chambers. By the fifth day, however, before the first cyanide dose was given the respiration appears to have been fairly steady. Considering average values for successive 3-day periods and expressing results as c.c./10 kg. fruit/hr. (Fig. 7), we shall not be far wrong if we estimate the initial respiration (either in c.c. oxygen uptake or CO_2 output) at 53 for the sample which was about to be treated with weak cyanide and at 46 for the controls at the same time. The departures from these initial values, which we shall term the *acceleration numbers*, during successive 3-day periods are recorded in Table 1.

The values of the acceleration numbers for the controls illustrate the conclusion drawn from the inspection of the charted values in Figs. 4 and 5 that both the CO_2 output and oxygen uptake remained steady until a late stage in phase (ii), when they tended to increase. In the other sample, before the first 3-day period of strong cyanide treatment was over, the value of the negative acceleration number for oxygen uptake, when related to the initial rate of 53, indicates that oxygen uptake had been depressed by nearly 40 %. This depression was maintained until the final phase, when in spite of oxidase browning it was enhanced. It is recalled that zymasis was a vigorous process during this period of depressed oxygen uptake. It is noteworthy that the acceleration numbers for CO_2 output were positive and greater than those

in the controls for most of the period when HCN zymasis was in progress, and were particularly high just before and just after the treated fruit was returned to pure air. Injuries to the apples had at this stage added to the complexity of the experimental system.

The results set out as in Fig. 7 and expressed in Table 1 as acceleration numbers indicate a little more clearly than do the points charted in Figs. 4 and 5 what were the drifts of CO_2 output and oxygen uptake after the cyanide dosing was increased. Moreover, they suggest that aerobic respiration had begun to decline under the influence of cyanide during the phase of weak cyanide treatment, when HCN zymasis, if occurring at all, only operated to a negligible extent. First, and most important, we note that the three acceleration numbers for oxygen uptake during this phase are negative. This is the only evidence we possess at present that cyanide may depress oxidative activity in apple tissue without inducing zymasis. The recorded values suggest that before the end of phase (i) the initial oxygen uptake had

Table 1. *Acceleration numbers representing departures, during 3-day successive periods, from the initial respiration rates of 46 c.c. oxygen uptake or CO_2 output per 10 kg./hr. for Newtown Pippin in air, and of 53 c.c. for fruit treated with weak* HCN *in phase* (i), *stronger* HCN *in phase* (*ii*), *and respiring subsequently in air in phase* (*iii*)

	Phase (i)	Phase (ii)	Phase (iii)
Air:			
CO_2 output	1, −1, 1	0, 2, 4, 1, 2	3, 4
Oxygen uptake	−2, −1, 1	2, −2, −1, 4, 4	3, 5
Air+HCN:			
CO_2 output	3, −1, −3	−3, 7, 4, 4, 10	25, 7
Oxygen uptake	−2, −9, −7	−18, −16, −18, −18, −19	−18, −23

been depressed by nearly 20 %. This fact marks the starting point for another experiment in which the amount of the depression brought about by HCN without the induction of zymasis will be compared with that produced by lowering the oxygen concentration in gas mixtures of nitrogen and oxygen around the tissues from 20 to 3 % (see p. 226). The results of this experiment will be reported in a later paper.

The evidence of a depression in oxidative activity without zymasis would have been more convincing had the acceleration numbers for CO_2 output during phase (i) and the beginning of phase (ii) been consistently negative and of similar magnitude to those for oxygen uptake, since, until HCN zymasis set in, the production of metabolic carbon dioxide by apple cells would result entirely from aerobic respiration. But it must be remembered that for several reasons some time may pass before changes in the rate of CO_2 production by cells in the interior of a bulky fruit are reflected in the changes measured in the gas composition of the atmosphere surrounding the fruit. Moreover, when the cyanide dosing was increased the tendency for CO_2 output to be depressed owing to the inhibition of oxidative processes would have been opposed by the increased production of CO_2 which accom-

panied the accumulation of organic products of HCN zymasis. The acceleration numbers suggest, however, that the minimum rate of CO_2 output was reached before HCN zymasis was occasioned during the early stages of phase (ii). This accords with our hypothesis (p. 226) that HCN zymasis is not induced until normal aerobic oxidative activity, relative to zymase cleavage, is depressed below a certain critical value.

3. The rise in the respiratory quotient during HCN zymasis

Increases above unity in the values of the R.Q.'s of plant tissues, brought about by changes in external or internal conditions, often indicate that anaerobic processes, such as zymasis, have begun to operate (see e.g. Stich, 1891; Genevois, 1927; van

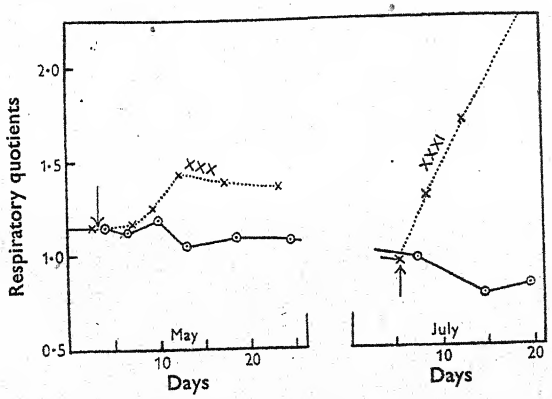

Fig. 6. The respiratory quotients of a sample (⊙) of Dunn's Favourite kept continuously in air and of samples (×) treated with moderate doses of HCN in May (XXX) and June (XXXI) 1932, on the dates indicated by the arrows. The cyanide numbers and alcohol numbers recorded below show that although senescence zymasis occurred in the apples ageing in air, the rate of increase in the alcohol number was much faster in apples treated with HCN.

	On 25 May		On 25 July	
	Cyanide number	Alcohol number	Cyanide number	Alcohol number
Air controls	0·1	56	0·1	129
Cyanide-treated apples	2·9	196	6·4	367

Raalte, 1937; James & Hora, 1940). From our measurements of the R.Q.'s of apples we may infer that HCN zymasis was quickly induced by cyanide treatment of Dunn's Favourite (Fig. 6). The rise in the R.Q. in the experiment on the more senescent apples was sudden and steep, and severe injuries were seen before the end of the experiment. Values obtained in determinations of final alcohol numbers verified this inference. Similarly verified evidence of HCN zymasis was secured in XXII on Granny Smith (Fig. 3), and after the stronger dosing of Newtown Pippin (Fig. 7) in XXIII.

We emphasize the point that whereas the detection of changes in R.Q.'s may often be the most delicate way of securing evidence of the energy of anaerobic

processes into metabolism, unambiguous evidence comes only from the measurement of changes in the concentration of ethyl alcohol, or of some other specific product of anaerobic metabolism. In superior experiments the accumulation of such specific products and gaseous exchanges are simultaneously measured. The experiments reported in this paper were among the first on the higher plants in which this superiority was aimed at. In some respects apple tissue has proved refractory, and some of our conclusions have still, perforce, to be tentative.

From the results discussed in § 2, we may, however, draw the general conclusion that the rise in the R.Q. in cyanide-treated apples was primarily owing to a reduction in the rate of oxygen uptake. James & Hora (1940) arrived at a similar conclusion

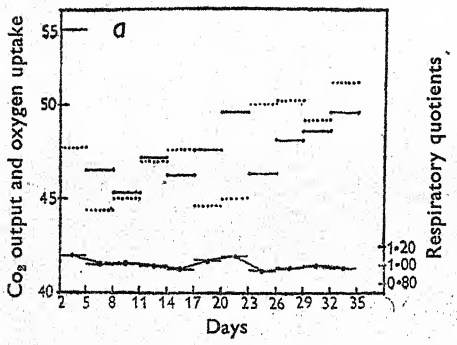

Fig. 7 *a*.

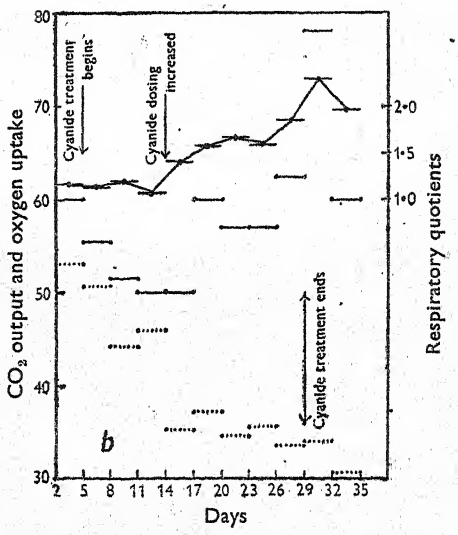

Fig. 7*b*.

Fig. 7 *a*, *b*. (i) Average rates of oxygen uptake (•···•) and CO_2 output (•—•) during successive 3-day periods of Newtown Pippin (XXXIII), expressed as c.c./10 kg. fresh weight per hour, and (ii) average R.Q.'s (—•—). Fig. 7*a*, data for a sample of 12 apples kept continuously in air. Fig. 7*b* data for a sample of 12 apples treated with HCN. Changes in the cyanide number and alcohol number are recorded under Figs. 3 and 4.

from the results of their experiments on the effects of cyanide on barley leaves. These investigators found, however, that the rate of CO_2 output might be simultaneously retarded, albeit to a less extent. They did not report any rise in CO_2 output such as we have encountered in most of our experiments in which HCN zymasis was a vigorous process. Genevois (1927) obtained results in his experiments on sweet-pea seedlings which indicated that the R.Q.'s of the seedlings were enhanced by treating them with HCN at concentrations above certain very low values. He stated that in *M*/10,000 HCN the CO_2 output increased without any change in the oxygen uptake. In the present paper we report no parallel to this finding. In concentrations of *M*/1000 HCN the oxygen uptake of the seedlings was often considerably retarded, while the CO_2 output was even higher than it was in *M*/10,000 cyanide, i.e. as in some of the experiments on apples described in the present paper,

two causes acted in concert in bringing about a rise in the R.Q. In those of our experiments in which the cyanide treatment was so strong or in which the variety used was so sensitive to cyanide that the CO_2 output was retarded, the greater retardation of oxygen uptake must have been responsible for the observed rises in the R.Q.

So far we have not succeeded in demonstrating that increases in the R.Q. brought about by cyanide treatment may be reversed by returning apples to pure air. We hoped to succeed in proving this point in XXII and XXIII, but HCN zymasis continued after the return of the fruit to pure air, and the apples suffered severe damage. James & Hora found that increases in the R.Q. induced by $M/100$ HCN were reversed by transferring barley leaves to water; but they did not cite evidence of the occurrence of zymasis during the period of cyanide treatment. In XIV, XV and XVI on Dunn's Favourite, reported in the preceding paper (p. 233), evidence of reversibility was obtained from measurements of ethyl alcohol and acetaldehyde production during cyanide treatment and after the return to air; but unfortunately gaseous exchanges were not measured. The crucial experiment has still to be performed.

4. The resolution of total CO_2 output during HCN zymasis into CO_2 produced in zymasis and CO_2 of persisting aerobic respiration

Carbon dioxide may be produced during HCN zymasis by at least two processes, viz. such aerobic respiration as persists,

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O, \qquad \text{(i)}$$

and such decarboxylation of pyruvic acid as leads to the accumulation of acetaldehyde and ethyl alcohol, i.e. to an increase in the alcohol number,

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH. \qquad \text{(ii)}$$

In XXIII on Newtown Pippin $CO_2.T.$ was directly measured in cyanide treated apples and in the controls. Values for the component parts of $CO_2.T.$ have been calculated (see p. 242) from the data charted in Figs. 5 and 6, and are recorded in Table 2. Taking the R.Q. of aerobic respiration as unity, $CO_2.O.R._{Air+HCN}$ has been evaluated from the measurements of oxygen uptake. $CO_2.Z._{Air+HCN}$ has been calculated from the measured increases in the alcohol number (z mg.) over the stated periods. From equation (ii), and taking the density of CO_2 at 0° C. as 1·98, we get

$$CO_2.Z._{Air+HCN} = \left[\frac{44 \times (273+t)}{46 \times 273 \times 1{\cdot}98}\right] z \text{ c.c. at } t° \text{ C.}$$

It is obvious that the value of any $CO_2.Res.$ in Air + HCN would be given by

$$[CO_2.T. - (CO_2.O.R. + CO_2.Z.)].$$

The records show that during the opening phase of 9 days the total CO_2 output and the oxygen uptake of the cyanide-treated samples and the control samples were nearly equal. We infer that the samples were sufficiently large to be considered comparable, and we shall assume that throughout the experiment the

control sample gave values that the treated samples would have given had they not been dosed with HCN.

As we have pointed out in previous sections, increases in the alcohol number and in the R.Q. for total respiration show that during the second phase of 15 days HCN zymasis was a vigorous process. The important point now emerges from the records in Table 2 that CO_2.Z. and CO_2.O.R. account for all the CO_2 produced in Air+HCN during this phase. There was no CO_2.$Res._{Air+HCN}$. This finding contrasts with the fact that under anaerobic conditions (p. 254) CO_2.$Res._{N_2}$ may be of considerable magnitude, and that HCN does not interfere with its production.

In spite of the fact that two apples out of the sample of twelve appeared to be sound at the end of the experiment, CO_2 seems to have been exclusively produced by zymasis during the final phase in pure air. The oxidation in disorganizing cells of polyphenols under the agency of catechol oxidase was presumably responsible for the oxygen uptake of the treated apples during this period.

Table 2. *The influence of* HCN *on the* CO_2 *output of Newtown Pippin in XXIII. Except in* (4) *the recorded values represent total volumes in c.c./*100 *g. fresh-weight tissue over the stated periods*

	Phase (i) 9 days		Phase (ii) 15 days		Phase (iii) 6 days	
	Air +HCN	Air	Air +HCN	Air	HCN treated apples in air	Air
(1) CO_2.T.	105	99·9	176	170	95·8	74·1
(2) Oxygen uptake	96·9	96·7	107	175	45·8	84·2
(3) CO_2.O.R. [calc. from (2)]	96·9	96·7	107	175	*	84·2†
(4) Increase in alcohol number in mg. resulting from HCN treatment	10	—	137	—	200	—
(5) CO_2.Z. [calc. from (4)]	5·4	—	69·6	—	101	—
(6) CO_2.Res.	2·7	—	Nil	—	Nil	—

* Since oxidase browning took place in most of the apples during this last phase, CO_2..O.R. cannot be calculated from (2). Inasmuch as CO_2.T. and CO_2.Z. are approximately equal for this period, we may infer that CO_2 production resulted entirely from zymasis.

† This value will be high because the ageing controls showed some oxidase browning.

5. THE EFFECT OF HCN ON THE RATE OF ANAEROBIC CO_2 OUTPUT

The course of the production of CO_2.N.R. in XXIV on Newtown Pippin (Fig. 8) in pure nitrogen was similar to that figured by Parija (1928) and Blackman (1928) for many of their experiments on Bramley's Seedling. It is clear from the charted values that cyanide treatment under anaerobic conditions led to a depression in the rate of total CO_2.N.R.

Consideration for treated and control apples (see Table 3) of the increases in the alcohol number during the two successive periods of 8 days (in which the cyanide number of the treated fruit increased to 2·2) and 14 days (at the end of which the cyanide number was 14·2) reveals that total CO_2.N.R. was not composed entirely of CO_2.Z., but that CO_2.Res. made a substantial contribution to it.

Using several varieties of apple, Fidler (1933*b*) obtained values ranging from 100/47 in an experiment on Wealthy to 100/97 in an experiment on Bramley's Seedling for the ratio CO_2.N.R./alcohol number. The difference between the numerator and denominator in this ratio will be slightly less than the percentage value of the CO_2.Res. fraction in CO_2.N.R. (see, for example, the last two lines of Table 3). In the experiment on Bramley's Seedling it is clear that CO_2.N.R. was composed almost entirely of CO_2.Z., i.e. nearly all of it was unquestionably of

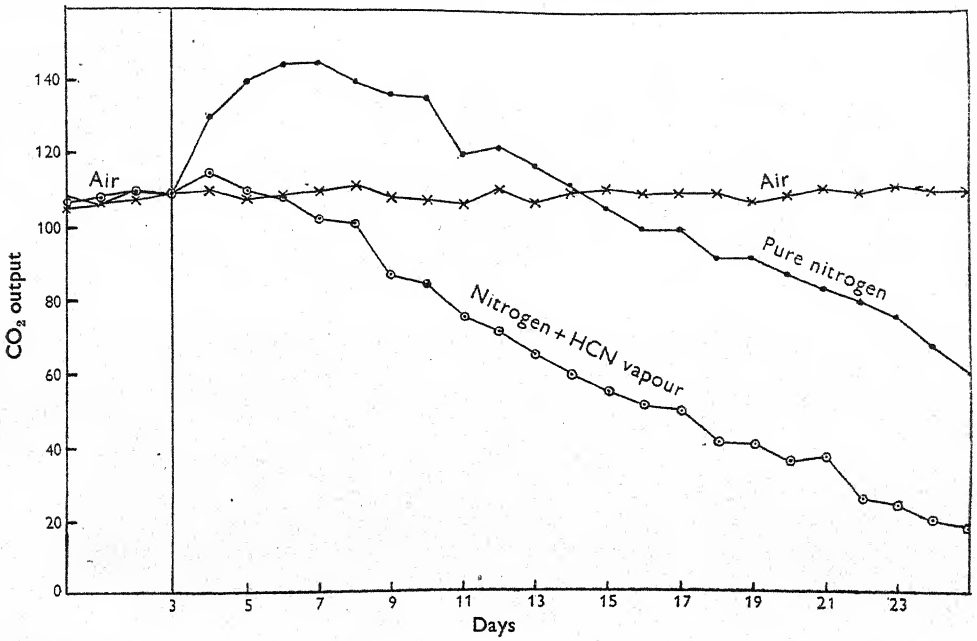

Fig. 8. The CO_2 output at 12° C., expressed as mg./10 kg. fresh weight per hour, by Newtown Pippin (XXIV) in air (×), pure nitrogen (•), and in nitrogen containing a moderate concentration of HCN (⊙). Samples of twelve apples each were used. The aerobic respiration during the opening three days shows that they were comparable. Values for the cyanide number and of such increases in the alcohol number as may be attributed to the exclusion of oxygen are recorded below. Values for the accumulation of acetaldehyde have been recorded in Table 6 on p. 237.

	After 8 days		After 14 days	
	N_2+HCN	N_2	N_2+HCN	N_2
Cyanide number	2·2	0·3	14·4	0·4
Alcohol number	110	206	182	452

metabolic origin, whereas in Wealthy over 50 % of CO_2.N.R. was CO_6.Res. The behaviour of Newtown Pippin in XXIV was intermediate between that shown by Bramley's Seedling and Wealthy in the earlier experiments.

In XXIV during each of the successive periods of 8 and 14 days, 75 % of $CO_2.N.R._{N_2}$ was $CO_2.Z._{N_2}$, and the remaining 25 % was $CO_2.Res._{N_2}$. It is not known where this residual CO_2 comes from, but the values after 8 and 14 days for

$CO_2.Res._{N_2+HCN}$ and $CO_2.Res._{N_2}$ indicate that HCN had not depressed the rate of production of $CO_2.Res.$ As the experiment proceeded $CO_2.Z._{N_2+HCN}$ became increasingly less than $CO_2.Z_{N_2}$, i.e. the inhibitory influence of cyanide was exerted on such zymase cleavage as resulted in the accumulation of acetaldehyde and ethyl alcohol.

Table 3. *The resolution of total* $CO_2.N.R.$ *produced in successive periods by Newtown Pippin at* 12° C. (*see Fig.* 4) *into* $CO_2.Z.$ *and* $CO_2.Res.$; *and data for the evaluation of the Pasteur effect. Values in total mg./*100 *g. fresh weight per period*

	8 days			14 days		
	N_2+HCN	N_2	Air	N_2+HCN	N_2	Air
$CO_2.O.R.$	—	—	209	—	—	370
$CO_2.N.R.$	189	262	—	144	312	—
Increase in alcohol number	110	206	—	72	246	—
$CO_2.Z.$ (calc.)	105	197	—	69	235	—
$CO_2.Res.$ (calc.)	84	65	—	75	77	—
$CO_2.N.R./$alcohol number	100/58	100/79	—	100/50	100/79	—
$CO_2.N.R./CO_2.Z.$	100/56	100/75	—	100/48	100/75	—

6. THE EVALUATION OF THE 'PASTEUR EFFECT' IN APPLES, INCLUDING NEWTOWN PIPPIN RESPIRING IN AIR AT 12° C.

The Pasteur effect in Bramley's Seedling

In living cells showing the Pasteur effect, the rate of carbohydrate catabolism bears some inverse quantitative relation to the rate of aerobic respiration, expressed in terms of $CO_2.O.R.$, oxygen uptake, or the equivalent oxidative consumption of hexose. It is obvious that the best evidence that this effect is or is not an operative factor in the metabolism of the higher plants would come from the results of experiments in which changes in carbohydrate concentration were measured in a sample living in air, or in a gas mixture of nitrogen and a known concentration of oxygen, and in a comparable sample living under anaerobic conditions. Inasmuch as there are so many possible sources of hexose in the higher plants, this method presents great difficulties. These no doubt will be overcome, but in the meantime, without measuring carbohydrates, solid evidence can be and has been obtained that the Pasteur effect operates in some of the higher plants (see Thomas, 1940). Data for the evaluation of the Pasteur effect in such plants are, however, scanty; but, as will be made clear below, approximate values may be sometimes determined if, in parallel experiments, measurements are made of the CO_2 output and oxygen uptake in air, and of the CO_2 output and increase in the alcohol number in pure nitrogen.

Blackman (1928) inferred from the fact that the R.Q. of Bramley's Seedling has a value of about unity, that $CO_2.O.R.$ comes exclusively from the oxidation of hexose, and that it is therefore a measure of the rate of catabolism of carbohydrates. He expressed hexose catabolism in the terms of the carbon units in $CO_2.O.R.$ He postulated that anaerobic respiration consisted entirely in zymasis, and inferred from this postulate that anaerobic catabolism would be $3CO_2.N.R.$, which he expressed in carbon units. His experimental values for $CO_2.N.R./CO_2.O.R.$

ranged from 1·3 to 1·5, which implied that hexose was consumed from about 4 to 4·5 times faster under anaerobic conditions than in air. Virtually he had evaluated the Pasteur effect, since it is an obvious deduction that for every one molecular equivalent of hexose oxidized in aerobic metabolism, from 3 to 3·5 molecular equivalents were conserved from catabolism.

Fidler (1933*b*), however, by making measurements over three seasons, found that on the average the ratio CO_2.N.R./alcohol number in Bramley's Seedling was 100/85, i.e. rather more than 15 % of the total CO_2.N.R. should be described as $CO_2.Res._{N_2}$ and was, as we have pointed out, of unknown origin.

Let us consider two of the possible ways of developing the argument when CO_2.Res. is an important fraction of total CO_2.N.R., for illustration assuming that we are dealing with a Bramley's Seedling in which the CO_2.N.R./CO_2.O.R. ratio is 1·5/1, and the CO_2.N.R./alcohol number ratio is 100/85, i.e. the CO_2.N.R./CO_2.Z. ratio is 100/81.

(i) We might confine our analysis to the results obtained from what are unquestionably catabolic processes involving hexose, namely, the production of CO_2.O.R. in air, and the increase in the alcohol number and the production of the equivalent CO_2.Z. in nitrogen. Then from the equations for aerobic respiration and zymasis given on p. 251 we see that had the rate of hexose catabolism in air been unity, that of hexose catabolism in nitrogen would have been $3 \times 1{\cdot}5 \times 81/100$. We could then have inferred that the oxidation of one molecular equivalent of hexose preserved 3·6 molecular equivalents from undergoing zymasis, i.e. conserved 2·6 molecular equivalents for future use by the tissue. It is manifest that in this measurement of the Pasteur effect we have been exclusively considering what are known to be metabolic processes. The suggestion of Boswell & Whiting (1940) that on transferring certain plant tissues from aerobic to anaerobic conditions, CO_2 may be liberated from chemical combination with cell substances has little bearing on the evidence for Bramley's Seedling as presented by Blackman, which has only been slightly modified in the light of Fidler's work.

(ii) The value obtained for the Pasteur effect by method (i) would be too low if $CO_2.Res._{N_2}$ also originated from the anaerobic oxidation of hexose. When $CO_2.Res._{N_2}$ is an important fraction of CO_2.N.R., Blackman's lead in expressing relative rates in terms of carbon units may be advantageously followed. The use of the term 'Pasteur effect' might then, for certain issues, have to be extended to cover events other than the catabolism of carbohydrates. In the experiment on Bramley's Seedling we may state that relative to a loss as CO_2.O.R. in air of 1 carbon unit, in nitrogen 1·5 units were lost as total CO_2.N.R., and 2·6 units in the alcohol number. We should then not go beyond the experimental evidence in concluding that when 1 carbon unit was lost as CO_2.O.R. in air, 3·1 units were preserved from undergoing catabolism with the production of ethyl alcohol and acetaldehyde or from being liberated as carbon dioxide (the greater part of which, at least, was of metabolic origin). The possibility is not excluded in this statement of additional catabolism of hexoses without the production of carbon dioxide and organic products of zymasis, or of the catabolism of other classes of metabolites.

The Pasteur effect in Newtown Pippin

The results obtained in XXIV enable us to calculate the magnitude of the Pasteur effect in the control sample of Newtown Pippin, living in air. Since $CO_2.Res._{N_2}$ contributed 25 % of the total $CO_2.N.R.$, the analysis will in the first place be made in terms of carbon units. The values tabulated below have been calculated from the data given for the first period of 8 days in Table 3 on p. 254:

	N_2	Air
C in $CO_2.O.R.$	—	57
C in alcohol number	107·5	—
C in $CO_2.Z.$	53·7	—
C in $CO_2.Res._{N_2}$	17·7	—
Total C loss	179	57

We conclude that the aerobic oxidation of 1 carbon unit is related to the conservation of 2·1 units in the sense given at the end of (ii) above.

Since we see that 90 % of the total carbon lost under anaerobic conditions in Newtown Pippin is found in $CO_2.Z.$ and the alcohol number, we may from the data given in Table 3 evaluate approximately the apparent conserving effect of oxygen on hexose catabolism, i.e. the Pasteur effect as defined at the beginning of this section. The argument develops along slightly different lines from those followed in considering the behaviour of Bramley's Seedling. There is as yet no unrivalled best method of analysis.

First we note that in the air controls, the R.Q. being about unity, $CO_6.O.R.$ was exclusively produced by the aerobic oxidation of hexoses according to equation (i) on p. 251; i.e. 1 mg. of $CO_2.O.R.$ was derived from the catabolism of 0·68 mg. of hexose. We point out in passing that it has never been proved that ripe apples catabolize hexoses without the production of CO_2.

Secondly, equation (ii) on p. 251 informs us that 1 mg. of $CO_2.Z.$ is derived from the catabolism of 2·05 mg. of hexose.

Accordingly, we deduce that during the opening 8 days of XXIV 142 mg. hexose were oxidized in apples living in air, while 404 mg. underwent anaerobic zymasis in pure nitrogen. We infer that the oxidation of 1 hexose unit in air was related to the preservation of 2·9 hexose units from undergoing zymasis, i.e. to the conservation of 1·9 hexose units for future use by the cells. If we assume that $CO_2.N.R.$ was produced by the complete anaerobic oxidation of carbohydrates, hexose conservation would have been 2·2 instead of 1·9.

For the results obtained in experiments on Bramley's Seedling, Newtown Pippin, and, indeed, in most varieties of ripe apples, the differences found by evaluating the Pasteur effect by the different methods we have described, are inconsiderable. The differences may become much more important when plant material has been used in which $CO_2.Res._{N_2}$ contributes a substantial fraction to total $CO_2.N.R.$, and when the ratio $CO_2.N.R./CO_2.O.R.$ is less than unity. Further discussion will

be deferred until the results are published of experiments that have recently been performed at Newcastle-upon-Tyne on respiratory catabolism in certain germinating seeds and green leaves.

7. Increased hexose catabolism during HCN zymasis considered as evidence of the Pasteur effect

A reasonable supposition is that such tissues as have been proved to show the Pasteur effect by experiments in which the rates of aerobic and anaerobic catabolism are compared, the rate of hexose catabolism should be increased when aerobic oxidations in the tissues are retarded by such poisons as HCN or H_2S, provided that the potential rate of anaerobic glycolysis (i.e. zymasis in apples) is not substantially reduced by the poison at the concentration used. The converse proposition is that the Pasteur effect is operative in at least some of those tissues in which hexose catabolism is increased under the influence of cyanide. It may sometimes happen that comparisons are made of tissues treated with cyanide and of air controls before anaerobic experiments are carried out. Suggestive, but not indisputable evidence may be got by such experiments.

In some, but not all, of the earlier experiments (§ 2) it was found that when apples were treated with HCN, the CO_2 output increased, while ethyl alcohol and acetaldehyde were accumulating in the tissue. Evidence was thus obtained that HCN may stimulate the rate of hexose catabolism. On the other hand, in experiments on some varieties (e.g. Beauty of Bath, p. 245) HCN so quickly depressed zymasis that the rate of hexose catabolism was always greater in the apples kept in air. Doubtless hexose catabolism in all varieties would be depressed if cyanide treatment were made sufficiently strong; and, probably, there exist cyanide conditions that would promote hexose catabolism in all varieties of apple, including Beauty of Bath, at least for a short period. Such conditions were clearly prevalent in XXXIII–XXXV on Newton Wonder (Fig. 1), and XXXVII and XXXVIII on Dunn's Favourite (Fig. 2).

The first evidence that the reduction of oxygen uptake goes hand in hand with the augmentation of hexose metabolism in cyanide-treated apples came from XXII on Granny Smith (Fig. 3 and Table 3). This experiment and some experiments on H_2S zymasis, which will be described elsewhere, gave information that was exploited in planning XXIII on Newtown Pippin.

We recall (§ 4) that we obtained no evidence in XXIII of the occurrence in apples treated with air and HCN of a process corresponding to that which contributes CO_2.Res. to the total CO_2.N.R. liberated by Newtown Pippins placed under anaerobic conditions. $CO_2.T._{Air+HCN}$ was resolved quantitatively into $CO_2.Z._{Air+HCN}$ and $CO_2.O.R._{Air+HCN}$. Further large-scale experiments would have to be performed to determine whether this result illustrated the exception rather than the rule; but it happens to place us in a position to calculate for this experiment the rate of such hexose catabolism (instead of catabolism in carbon units) as led to the production of CO_2 in the cyanide-treated apples. In air hexose catabolism can be calculated from CO_2.T., which was of course wholly CO_2.O.R.

In Table 4 the values are recorded of the hexose equivalents of the CO_2 outputs in the cyanide treated Newtown Pippin and the air controls for the whole of each of the three phases of XXIII. The basis for the calculations has been given on p. 256.

The recorded values show that the average daily rate of hexose catabolism in the air controls remained steady during the course of the experiment, and that during the opening phase of 9 days there was little difference between the behaviour of the cyanide-treated apples and the controls. In striking contrast we find that this rate was nearly doubled during the second phase when HCN zymasis had become a vigorous process, and it is a significant fact that at the same time the rate of oxygen uptake was greatly reduced. The great intensification of hexose catabolism during the final phase, when its average rate reached the high value of 65, cannot be entirely attributed to reduced aerobic respiration, which may have been completely suppressed (p. 252), since decreased organization resistance in the injured cells

Table 4. *Data based on the results recorded in Figs. 3 and 4, or calculated for the evaluation of the Pasteur effect in Newtown Pippin (XXIII). The values record total mg./100 g. fresh weight per period*

	Phase (i) 9 days		Phase (ii) 15 days		Phase (iii) 6 days	
	Air+HCN	Air	Air+HCN	Air	Air+HCN	Air
CO_2. T.	197	188	331	320	181	139
Increase in alcohol number	10	—	137	—	200	—
CO_2. Z. (calc.)	10	—	131	—	190	—
CO_2. O.R.*	187	188	200	320	Nil	139
Oxygen uptake	126	125	139	227	60*	109
Hexose catabolism (calc.)	147	128	405	218	380	95
Average daily rate of hexose catabolism	16	14	27	15	65	16

* Since oxidase-browning contributed to the oxygen uptake during the final period, CO_2. O.R. has been obtained from the difference (CO_2. T.–CO_2. Z.).

may have been a major contributory cause. Our general conclusion, however, is that by these experiments we have obtained strong supporting evidence that the 'Pasteur effect' is one of the factors governing hexose metabolism in apples.

The quantitative relations that existed between aerobic respiration and the catabolism of hexose during the second phase of XXIII become clearer if values found or calculated in mg. are converted into mg. mol. by dividing them by the molecular weights of the substances concerned.

The positive fact then emerges (see Table 5) that in this experiment the depression by cyanide of the oxidation of one molecular equivalent of hexose, which implies a corresponding lowering by six molecular equivalents of the oxygen uptake, was related to the zymasis of 3·2 molecular equivalents of hexose, and, accordingly, to a net increase in hexose catabolism of 2·2 molecular equivalents.

If the depression in oxygen uptake was of a kind similar to that which occurs when the surrounding oxygen concentration is lowered (p. 266), and if cyanide treatment did not inhibit or stimulate the activity of the zymase complex, it is highly probable that these quantitative relations permit us to evaluate the magnitude

of the Pasteur effect in the aerobic metabolism of carbohydrates at 12° C. in the Newtown Pippin apples used in XXIII. A sufficient statement in terms of molecular units is that oxidation of one hexose unit by the fruit living in air was preserving 3·2 hexose units from undergoing zymasis of the anaerobic kind, and so conserving in the flesh tissue 2·2 hexose units. The agreement with the value obtained from comparing the rates of aerobic and anaerobic metabolism in pure nitrogen is close (p. 256).

Table 5. *Oxygen uptake and hexose catabolism expressed as mg. mol./*100 *g. fresh weight during* 15 *days. The values have been calculated from the data given for the second period in Table* 4

	Air+HCN	Air	Change in rate caused by HCN	Relative change
Oxygen uptake	4·34	7·09	−2·75	−6
Hexose equivalent of oxygen uptake	0·72	1·18	−0·46	−1
Hexose equivalent of CO_2.Z.	1·49	—	+1·49	+3·2
Hexose equivalent of CO_2.O.R.	0·76	1·21	−0·45	−1
Total hexose catabolism	2·25	1·21	+1·04	+2·2

Summary

1. The principle is stressed that when anaerobic glycolysis, in the form of zymasis, contributes to the total catabolism of carbohydrates by plant tissue, the scope of theoretical analysis is advantageously enlarged if, in addition to measuring CO_2 output and, under aerobic conditions, oxygen uptake, changes in the alcohol number are simultaneously determined.

2. These three magnitudes have been simultaneously measured in apples placed under conditions that induced HCN zymasis, and, also in control samples kept continuously in pure air. In the latter, CO_2 is believed to be produced exclusively from the normal aerobic respiration of hexose. When HCN zymasis was in progress in our experiments the total CO_2 output has been resolved into the CO_2 fractions produced in zymasis and in such aerobic respiration as persisted.

3. It has been proved that the accumulation of acetaldehyde and ethyl alcohol in HCN zymasis may be associated with a reduction in the rate of oxygen uptake, and, consequently, of the production of CO_2 by aerobic respiration.

In one instance a little evidence was obtained that aerobic respiration may be reduced by the action of HCN before zymasis sets in. This finding, if confirmed, would accord with the theoretical view (see p. 226) that HCN zymasis will not begin to operate until oxidative activity has been depressed by HCN at least as much as it would be when, in the absence of cyanide, there remains only 3 % oxygen in the atmosphere outside the fruit.

4. HCN zymasis is always accompanied by a rise in the respiratory quotient, which, in the typical case, may be attributed to a rise in the CO_2 output as well as to a reduction in the oxygen uptake. When HCN depresses CO_2 output while inducing HCN zymasis, it depresses oxygen uptake still more.

5. Special importance is attached to the discovery that during HCN zymasis, while aerobic respiration is depressed, the rate of total CO_2 output, compounded of zymasis CO_2 and of CO_2 produced by persisting aerobic respiration, may be increased. It is inferred that, by inhibiting oxidative activity, HCN may stimulate hexose catabolism. Evidence has thus been obtained of the operation of a 'Pasteur effect' in the normal aerobic metabolism of carbohydrates in apples. From the quantitative data recorded for an experiment on aerobic HCN zymasis in Newtown Pippin it has been calculated that the aerobic oxidation of 1 molecular equivalent of hexose in an apple living in pure air preserves 2·2 molecular equivalents of hexose from undergoing catabolism.

6. Measurements have been made of the anaerobic CO_2 output, and the simultaneous increase of the alcohol number, in Newtown Pippin apples placed in pure nitrogen; and the influence of HCN on anaerobic processes has been determined. The metabolic process of zymasis was responsible for about 75 % of the CO_2 produced under anaerobic conditions. The origin of the residual fraction of anaerobic CO_2 is unknown. By depressing anaerobic zymasis, HCN brought about a reduction in the rate of anaerobic CO_2 output; but it did not appreciably affect the rate of production of residual anaerobic CO_2.

7. It is asserted that the Pasteur effect may be evaluated in apples from the quantitative data obtained in collateral experiments by the measurements under aerobic conditions of the CO_2 output, and, preferably, of the oxygen uptake also, and under anaerobic conditions of the CO_2 output and changes in the alcohol number. Data obtained in earlier experiments on Bramley's Seedling have been discussed anew. From fresh data published in this paper we have calculated that the oxidation of 1 molecular equivalent of hexose by Newtown Pippin living in air, according to one set of assumptions, preserves 1·9 molecular equivalents of hexose from undergoing catabolism, or, according to another set, 2·2 molecular equivalents. The agreement with the value recorded in (4) is close.

Note by M. T. For two years (1931–3) this research was carried out under the auspices of the Department of Scientific and Industrial Research, as Dr J. C. Fidler then held the appointment of Research Assistant. He performed most of the analytical work described in Zymasis VIII and IX, but bears no responsibility for any of the conclusions that have been drawn from them.

Generous grants to meet the cost of material and apparatus have been received from the Royal Society and from the Research Grants Committee of Armstrong College, Newcastle-upon-Tyne.

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CARBOXYLASE AND COCARBOXYLASE IN BARLEY

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The presence of carboxylase in barley powders prepared by rapid drying and grinding has been shown previously by James & Norval (1938). Further information about the enzyme, especially its separation into an apo- and co-enzyme, is reported in this paper.

Methods

Sap and other preparations described below, together with additional substances to serve as substrates and a few crystals of thymol as antiseptic, were placed in incubation tubes. These wide tubes were fitted with a rubber bung carrying finer inlet and outlet tubes with screw clips, as used by James & Norval in their original experiments. The atmosphere of the tube was then replaced with nitrogen by exhausting, refilling with nitrogen bubbled through the sap or other preparation, and repeating this process a second and a third time. The commercial nitrogen used was freed from oxygen and carbon dioxide by passing it through three Pettenkofer tubes filled with alkaline pyrogallol. The clips were then closed and the tube shaken in a bath at 30° C. for the experimental period of 3 hr. This period was taken from the moment of closing the exhaustion flask after the last exhaustion, to the moment of opening the tube for the CO_2 determination. It was not possible to ensure that this period was always precisely 180 min., but the period was accurately timed, and if it exceeded 185 min. a proportional correction was made in the results. Below this time, the correction involved (5 %) was less than the overall accuracy of the method.

At the end of the experimental period, CO_2 was measured by the micromethod of James & James (1936). A CO_2-free air stream (passed through a soda-lime tower and a baryta tell-tale) is drawn through the liquid by means of an evacuated suction flask containing 25 ml. of $N/150$ baryta, and the CO_2 absorbed by the baryta is determined by titration with $N/150$ HCl.

For the present series of experiments the method was varied at two points. First, the liquid in the tube was strongly acidified before the estimation of CO_2, by allowing 2–3 ml. of 2 % phosphoric acid to be drawn into it from a 10 ml. graduated pipette. This was inserted into the inlet tube, before connection was made for the flushing air stream. The possibility of retention of CO_2 by the liquid was thus reduced. The addition of acid in this way also enables the first slight opening of the tube to be detected immediately by the movement of the liquid in the pipette, and so aids materially in preventing a sudden rush of air which otherwise

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may ruin a determination by carrying baryta from the tell-tale into the tube, or material from the tube into the suction flask.

Secondly, capryl alcohol is added to stop frothing. This is done by adding 3 ml. capryl alcohol to 100 ml. stock 2 % phosphoric acid, which is then vigorously shaken before the acid is taken up into the pipette.

As a precaution against gas leaks, it was found advisable to seal all permanent rubber-to-glass joints with collodion and to smear all stoppers lightly with vaseline before fitting. The rubber pressure tubing used to join the incubation tubes into the titration apparatus was similarly treated. All the glass parts, such as the burette, incubation tubes, gas inlet and outlet tubes and especially the suction flask in which the titration is finally carried out, must be cleaned regularly with hot chromic acid. Failure to do this soon results in loss of accuracy. Using the precautions named, determinations on duplicate tubes containing aliquots of a given preparation usually agreed within 0·1 ml. *N*/150 HCl. Purely chemical duplicates, as in the determination of titration blanks, checked to within one part in a thousand. Under these circumstances, the method could be used over a range of 0·03 —→ 1·0 mg. CO_2 with 5 % accuracy at 0·3 mg. The pulpy consistency of some of the preparations used made manometric methods unsuitable, but did not affect the accuracy of the method employed.

Glass-distilled water was used both for final washings of the apparatus and for all experiments.

The use of thymol. In spite of the relatively short incubation period of 3 hr., satisfactory duplicates could not always be obtained without the use of thymol as antiseptic. The addition of thymol only slightly reduced the amount of CO_2 obtained from the tubes, showing, in agreement with the experience of James & Norval (1938), that action by micro-organisms over such a period was small. The use of thymol in the present series of experiments did somewhat reduce the experimental error. No reason was found to suppose that it interfered in any way with with enzymatic reactions being examined.

Material

Barley sap was prepared from green seedling plants, 12–14 days old and 6–7 in. high. These were cut in bulk and in the majority of experiments were wrapped in small lots in muslin and frozen solid in the refrigerator at temperatures down to − 12° C. They were then thawed out at room temperature and pressed out by hand through muslin. The sap so obtained was light brown to yellow in colour and slightly turbid. It was left overnight in a tall cylinder at about 2° C. when slight sedimentation occurred and the sap became quite transparent although still retaining its colour. This sap was used without further treatment.

In certain later experiments sap was prepared by grinding the young plants to a pulp in a mechanical pestle and mortar and pressing it out through muslin. This yielded a dark green liquid which slowly deposited a bulky green precipitate after some hours' standing, or after a period of centrifuging, leaving a clear brown or yellow liquid. This sap was either shaken up and used raw, or else was centrifuged

free from the green solids. No qualitative difference was noticed in the experimental behaviour of the sap whichever of these procedures was adopted, but the pulped sap appeared, when fresh, to be more active than that obtained by freezing. This difference was reduced by keeping.

Thymol, at the rate of 0·3 g./100 ml., was added to all liquid preparations from barley, to minimize deterioration on keeping.

RESULTS

Carboxylase activity of frozen sap

The CO_2 emission of sap pressed from thawed barley is low, ranging from 0·03 to 0·15 mg./5ml./3 hr.

Table 1, column 3 gives figures of several estimations with different samples of sap.

Table 1. CO_2 *emissions in mg./5 ml./3 hr. of frozen barley sap, with and without pyruvic acid*

Exp. no.	Mg. pyruvic acid added	Emission without pyruvic acid	Emission with pyruvic acid
1 (fresh sap)	—	0·14	—
2 ,,	—	0·08	—
3 ,,	—	0·15	—
4 ,,	50·0	0·03	0·13
5 ,,	10·0	0·12	0·20
6 (sap kept 5 days)	15·0	0·08	0·09
7 (sap kept 6 days)	15·0	0·08	0·08

(All CO_2 figures in the above table represent the mean of closely checking duplicates.)

The increase of activity on addition of pyruvic acid (columns 3 and 4) is variable in amount, but is always observed in freshly prepared sap. It falls off considerably on keeping, and sap stored for 5 or 6 days in the refrigerator (experiments 6 and 7) showed no increase over the spontaneous CO_2 emission rate when pyruvic acid was added. It is probable that the carboxylase system itself had broken down on standing. It could not have been already saturated with pyruvic acid in the sap itself, since these saps have been shown to contain no appreciable amount of the acid (James *et al.* 1941). An unduly prolonged period of freezing of the barley during the preparation of the sap also appears to have a slight adverse effect on its enzymes, though it is impossible to particularize which enzyme is affected, since CO_2 emission is reduced both with and without addition of pyruvic acid.

Table 2. *Milligrams* CO_2 *emitted by 5 ml. sap + 0·2 g. barley powder in 3 hr. at* 30° C.

Duration of freezing in hr.	Without pyruvic acid		With 15 mg. pyruvic acid	
	Digest A	Digest B	Digest C	Digest D
10	0·27	0·27	0·45	0·44
24	0·20	0·21	0·42	0·41

The carboxylase activity of barley powder

The low CO_2 emissions observed in the experiments with sap + pyruvic acid suggested that the extraction of the carboxylase system was very incomplete. There was even a possibility that the pressing out of the sap might have brought out a soluble prosthetic group (cocarboxylase), leaving the remainder of the system (apo-enzyme) wholly or partly in the solid residue.

In powder prepared from leaves without the removal of anything but water, the system should be complete and its full activity able to be developed. Such a powder was prepared by drying young green leaves rapidly in a fast air stream warmed to 30° C. As soon as the leaves were brittle they were ground in a mechanical mortar to a fine powder which was suspended in glass-distilled water during incubation. The amount of fresh barley which yielded 5·0 ml. of sap gave approximately 0·5 g. of this powder, and these quantities of sap and powder are therefore treated as equivalent. The carbon dioxide emissions of sap and powder simultaneously prepared from a single picking of barley leaves, are shown in Table 3.

Table 3. *Milligrams* CO_2 *emitted in* 3 *hr. at* 30° C.

	Without pyruvic acid			With 15 mg. pyruvic acid		
	A	B	Mean	C	D	Mean
0·3 mg. powder + 5 ml. H_2O	0·17	0·17	0·17	0·29	0·27	0·28
5 ml. sap	0·09	0·06	0·08	0·09	0·10	0·10
0·5 g. powder (calculated, ≡ 5 ml. sap)	—	—	0·28	—	—	0·47

It is clear, therefore, that the powder is very much more active in releasing CO_2 than the sap, both from its own contained substrates and from the added pyruvic acid (= carboxylase activity).

Cocarboxylase

The separation of a soluble co-enzyme (cocarboxylase) and an insoluble apo-enzyme ('protein') was achieved as follows: Seedling barley was frozen, thawed and its sap pressed out in the usual way. The solid residue was washed out with five times its own weight of water and the washing repeated four times in all. The four extracts thus obtained were combined and reduced to a small volume by evaporation under reduced pressure and then added to the sap. The washed residue

Table 4. *Milligrams* CO_2 *emitted in* 3 *hr. at* 30° C. *from* 15 *mg. pyruvic acid*

	A	B	Mean
5 ml. sap	0·06	0·06	0·06
0·2 g. washed powder suspended in 5 ml. glass-distilled water	0·08	0·09	0·09
Sum	0·14	0·15	0·15
0·2 g. powder suspended in 5 ml. sap	0·18	0·21	0·20

was dried in a rapid air stream at 30° C., ground and sieved through muslin. Three digests were set up in duplicate. The first pair contained 5 ml. sap; the second 0·2 g. of the washed powder suspended in 5 ml. glass-distilled water; and the third the same amount of powder suspended in 5 ml. sap. 15 mg. pyruvic acid were added to each digest as a substrate for carboxylase activity.

The sap and powder when incubated together gave off substantially more CO_2 than the sum of their emissions when incubated apart. In other words, there was at least a partial separation of a soluble cocarboxylase in the sap and washings, from a necessary apo-enzyme left in the powder.

The action of cocarboxylase from yeast

Yeast cocarboxylase in solution[1] was first tested in a washed yeast suspension and found to be active in increasing its CO_2 output. Further aliquots of the same preparation were then applied to barley as follows: Sap was pressed out from frozen barley in the usual way and the residue rapidly dried and ground. Part of the powder was then given two rapid washings with $N/10$ Na_2HPO_4 followed by one with distilled water, to complete the removal of the barley cocarboxylase. This procedure greatly reduced the powder's carboxylase activity, which was only very partially restored by an addition of the yeast cocarboxylase.

Table 5. *Milligrams* CO_2 *emitted in* 3 *hr. at* 30° C. *from* 15 *mg.* Na *pyruvate*

	A	B	Mean
0·5 g. unwashed powder + 12 ml. water	0·26	0·26	0·26
0·5 g. *washed* powder + 12 ml. water	0·12	0·12	0·12
0·5 g. *washed* powder + 12 ml. water + 10γ cocarboxylase	0·15	0·15	0·15

It was thought that the failure to restore full activity by the addition of yeast cocarboxylase might result from damage to the barley apo-enzyme by the alkaline washings. The conditions were, therefore, varied by using only a single brief alkaline washing on dried seedling leaves, thus omitting the freezing and pressing and one alkaline washing. The alkaline washing was followed by a distilled water washing as in the previous experiment and a similar series of digests was set up. While a similar decline of activity was found to follow the washing no restoration of activity could be brought about in this case by the addition of yeast cocarboxylase, possibly owing to a deficiency of Mg or other divalent metals. We are therefore unable without further work to compare the identities of the yeast and barley co-enzymes.

The thiochrome test for cocarboxylase

The presence of a cocarboxylase in barley sap was next successfully investigated by means of the thiochrome reaction. 70 g. barley seedlings, grown for 14 days in the dark to prevent the formation of the pigments of the chlorophyll com-

[1] Given to us by Prof. R. A. Peters to whom we are greatly indebted.

plex, were frozen and thawed out and the sap expressed. The sap was neutralized to litmus with barium hydroxide solution (0·35 *N*) and brought rapidly to the boil for a few seconds. It was then cooled and filtered and a drop of saturated ammonium sulphate solution was added, to remove excess barium. The precipitate of barium sulphate was removed on the centrifuge, and the clear yellow liquid thus obtained was examined in the light of a screened mercury-vapour lamp after oxidation by alkaline ferricyanide. The existence of the oxidation product with blue fluorescence was observed. The writers are indebted to Mr Kinnersley, of the Department of Biochemistry, for the estimation of the amount of cocarboxylase present; he found the equivalent of 30γ cocarboxylase per 100 g. original tissue.

Summary

1. The carboxylase activity of barley powders was confirmed, and a smaller activity demonstrated in clear saps.

2. The activity of a mixture of sap + residual powder was greater than the sum of their separate activities. From this it was concluded that the carboxylase system of barley includes an insoluble apo-enzyme and a soluble co-enzyme, like that of yeast.

3. Cocarboxylase from yeast did not fully restore the activity of washed barley powders.

4. Clear barley saps gave a positive fluorescence test for cocarboxylase. This method yielded an estimate of 30γ (as diphosphothiamine) per 100 gm. of original barley tissue.

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ON THE MECHANISM OF GLYCOLYSIS IN BARLEY

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An outline of the path by which sugars are broken down by barley has previously been suggested (James, 1938). A nodal position was assigned to pyruvic acid, and subsequent work has shown the convenience of giving it special attention. The existence of a carboxylase system, breaking it down to carbon dioxide and acetaldehyde, was first shown by James & Norval (1938) and has been confirmed in the paper preceding this. The importance of phosphorylation has also been shown (James & Arney, 1939), and some of its more important reactions identified (James *et al.* 1941). The work described in the present paper has provided some new and confirmatory data, with the result that one can now formulate a scheme with considerably more detail (p. 272) than was possible in 1938.

Methods

The experiments of the preceding paper (Bunting & James, 1941) showed that barley saps and powders contained a carboxylase system which is not saturated by the substrates also unavoidably present. Any substance capable of giving rise to pyruvic acid when added to such preparations, should, therefore, increase their CO_2 output. We interpret such increases of CO_2 as showing that the substance added is catabolized, and probably through the formation of pyruvic acid. Where it has previously been shown that pyruvic acid may finally be isolated from the digest, if the carboxylase is poisoned (as in the experiments of James *et al.* 1941), this intermediate stage seems clear. The advantage of the CO_2 method lies in the facts that CO_2 is more easily identified and more accurately estimated than pyruvic acid, particularly in the presence of barley residues. In effect, the present paper confirms the results of James *et al.* by showing that every substance which gave isolatable pyruvic acid also gave the expected increase of carbon dioxide.

The methods for carbon dioxide estimation, incubation of digests and preparation of saps and powders were used exactly as described before (Bunting & James, 1941).

Results

Adenylic acid

In saps treated with 1-naphthol-2-sulphonic-acid, to poison their carboxylase, adenylic acid was found to give an increased formation of pyruvic acid (James *et al.* 1941). Since adenylic acid is soluble and diffusible it is likely to become dispersed in cell-free preparations, so that further additions will be needed to restore the effective concentrations at the phosphorylating centres. The smaller activity of saps

when compared with powders (cf. previous paper) may be partly ascribed to a greater degree of dispersion of their co-enzymes and carriers. If this is so, the saps should be particularly favourable material for the study of adenylic acid additions. In the present series of experiments, sap was prepared in the usual way, and aliquots were incubated for 3 hr. at 30° C. with the additional substances shown in Table 1. The adenylic acid was a sample provided by British Drug Houses.

Table 1. *Milligrams* CO_2 *emitted by* 5 *ml. barley sap in* 3 *hr. at* 30° C.

	A	B	Mean
5 ml. sap	0·12	0·12	0·12
5 ml. sap + 1 mg. adenylic acid	0·23	0·18	0·21
5 ml. sap + 10 mg. pyruvic acid	0·18	0·20	0·19

Columns A and B in this and succeeding tables give the results of duplicated incubations.

The addition of adenylic acid greatly increased the output of CO_2, apparently even up to the limit set by saturation of the carboxylase system. The high efficiency of the adenylic acid was shown in another way also. If the extracted residue is dried, ground and added back to the sap, the rate of CO_2 output is much increased over that from sap alone, but the addition of a much smaller quantity of adenylic acid caused an even greater CO_2 output. In this experiment, CO_2 emissions were not limited by the carboxylase activity (bottom line in Table 2) which was much greater than in the previous set.

Table 2. *Milligrams* CO_2 *emitted by* 5 *ml. barley sap in* 3 *hr. at* 30° C.

	A	B	Mean
5 ml. sap + 200 mg. dried residue	0·27	0·27	0·27
5 ml. sap + 1 mg. adenylic acid	0·35	0·35	0·35
5 ml. sap + 10 mg. pyruvic acid	0·44	0·45	0·45

This experiment was repeated on the following day with further aliquots from the same preparations of sap and powder (= extracted residue). The results were strikingly similar, as is seen by comparing Tables 2 and 3.

Table 3. *Milligrams* CO_2 *emitted by* 5 *ml. barley sap in* 3 *hr. at* 30° C.

	A	B	Mean
5 ml. sap + 200 mg. dried residue	0·24	0·29	0·27
5 ml. sap + 200 mg. dried residue + 1 mg. adenylic acid	0·35	0·35	0·35

In an experiment with sap pressed from barley freshly pulped without freezing, the CO_2 output was high, reaching the same value of 0·35 mg. CO_2/3 hr.; and there was no further increase on adding 1 mg. adenylic acid. We were not, however, able to perform further experiments to decide whether this really represented the saturation point of the system for adenylic acid. The more important point for our purpose, that when the CO_2 output is initially low it may be raised by adding adenylic acid, seems sufficiently established. In one respect these results differ

from those obtained previously. In order to get enough pyruvic acid for a satisfactory isolation, it was also necessary to add additional sugar to the saps. That this was not needed in the present experiments was probably largely due to the greater sensitivity of the CO_2 method. In addition, no steps were taken to reduce the natural sugar content of the saps as in the isolation experiments, since it was not thought necessary to demonstrate that the sugars themselves are converted to carbon dioxide.

Hexosediphosphate

Weighed barium hexosediphosphate was dissolved in a small amount of water, slightly acidified with HCl. The barium was precipitated by the addition of one or two drops of saturated ammonium sulphate solution, and centrifuged off. The solution was neutralized (purple litmus) with NaOH and made up to volume. It was kept in the refrigerator and used within 24 hr. Solutions prepared in this way were used in a series of experiments with different preparations of sap and powder. In every case a large increase in the CO_2 output resulted from the hexosediphosphate addition. The individual results are shown in Table 4.

Table 4. CO_2 *emitted by barley sap in* 3 *hr. at* 30° C.

	Mg. hexosediphosphate added (as barium salt)	Mg. CO_2 emitted					
		Without hexosediphosphate			With hexosediphosphate		
		A	B	Mean	A	B	Mean
5 ml. sap	20	0·12	0·14	0·13	0·18	0·18	0·18
5 ml. sap	20	0·08	0·08	0·08	0·15	0·18	0·17
5 ml. sap	15	0·15	0·15	0·15	0·29	0·30	0·30
5 ml. sap*+0·2 g. extracted residue	10	0·20	0·24	0·22	0·35	0·35	0·35
5 ml. sap*+0·2 g. extracted residue	10	0·18	0·18	0·18	0·29	0·30	0·30

* These saps were pressed from fresh pulp without previous freezing.

Phosphoglycerate

A pure preparation of the acid barium salt was given to us by Prof. R. A. Peters, to whom we wish to express our gratitude. It was brought into solution in the same way as the barium hexosediphosphate, and the equivalent of 10 mg. acid barium salt was added to a mixture of fresh sap and barley powder capable of high carboxylase activity. The CO_2 output (Table 5, line 2) was raised virtually to the same level as by hexosediphosphate (Table 5, line 3) agreeing also with the comparable experiments shown in the last two lines of Table 4 above.

Table 5. *Milligrams* CO_2 *emitted by* 5 *ml. barley sap* in* 3 *hr. at* 30° C.

	A	B	Mean
5 ml. sap*+0·2 g. extracted residue	0·20	0·24	0·22
5 ml. sap*+0·2 g. extracted residue +phosphoglycerate	0·32	0·33	0·33
5 ml. sap*+0·2 g. extracted residue +hexosediphosphate	0·35	0·35	0·35

* These saps were pressed from fresh pulp without previous freezing.

The fact that phosphoglycerate and hexosediphosphate when added in relatively large amounts give rise to approximately the same rates of CO_2 emission, is important if we are to suppose that the sugar phosphate is broken down via phosphoglycerate. If the phosphoglycerate gave only slower rates of CO_2 emission, its position as an intermediate would have to be regarded as doubtful. So far as our experiments have yet gone, the required condition is fulfilled. The addition of pyruvic acid to similar digests led to even faster CO_2 rates, viz. 0·53 and 0·66, mean 0·60 mg. CO_2/3 hr. This permits the assumption that the following reactions occur in the order stated:

$$\text{hexosediphosphate} \longrightarrow \text{phosphoglycerate} \longrightarrow \text{pyruvic acid} \longrightarrow CO_2.$$

Sodium fluoride poisoning

The enzymic conversion of phosphoglycerate to pyruvate is known to be exceptionally sensitive to poisoning by sodium fluoride. Advantage may be taken of this fact to confirm the position of phosphoglycerate as an intermediate in the conversion of hexosediphosphate to pyruvate. We have already been able to show under favourable conditions that *M*/40 NaF inhibits the formation of pyruvate as estimated by Straub's *o*-hydroxybenzylidene-pyruvate method (James *et al.* 1941). It was hoped that the greater accuracy of the CO_2 estimation would allow this inhibition to be more fully investigated, but a complication arose when it was found that the fluoride itself released appreciable quantities of CO_2 from the barley preparations. The total CO_2 released in the presence of *M*/40 NaF, without addition of hexosediphosphate, had therefore to be taken as control value, and this proved to be rather large. Digests were set up in the usual way with barley material already known to be active in decomposing hexosediphosphate (Table 4, bottom line). The presence of fluoride thus completely inhibited the formation of additional CO_2 from the added hexosediphosphate. This result is in sharp contrast with

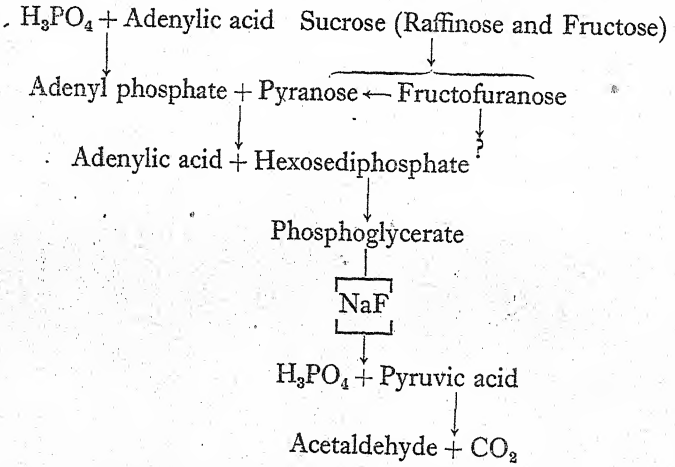
Table 6. *Milligrams* CO_2 *emitted by* 5 *ml. barley sap* +0·2 *g. extracted residue in* 3 *hr. at* 30° C.

	A	B	Mean
With *M*/40 NaF	0·36	0·36	0·36
With *M*/40 NaF + hexosediphosphate (≡10 mg. Ba salt)	0·37	0·35	0·36

those of Table 4, where no fluoride was present. One comment is necessary. The control value without hexosediphosphate is already rather high, and it might appear at first sight that the reason why no additional CO_2 was formed was that the carboxylase system was already saturated in the control. It was found, however, that digests, using similar unfrozen sap and powder, released 0·60 mg. CO_2 when pyruvic acid was added to them. They, therefore, possessed a comfortable margin of unsaturated carboxylase to decompose any pyruvic acid, had it been formed in the NaF experiments.

DISCUSSION

It was earlier suggested (James, 1938) that glycolysis in barley entailed the phosphorylation of glucose or fructose by means of a phosphate carrier described as a *labile ester*. After picking up the readily transferred phosphate of this ester, the sugars were said to form *resistant esters*[1] which were degraded via pyruvic acid to acetaldehyde and CO_2. Work carried out since has shown that the role of labile ester may be carried out by an adenyl phosphate (adenylic acid + H_3PO_4 + barley sap). Evidence has also accumulated which shows that hexosediphosphate and phosphoglycerate are two of the 'resistant esters'. As a result, the 1938 tabulation may now be expanded into the following:



The evidence for the existence of the above series of reactions in barley digests is contained in the present paper and in other papers quoted in the introduction. A few words as to how much the tabulation is intended to convey may be added here.

In general, what has been shown is that such reactions are catalysed by barley enzymes; and it is worth pointing out that, as it is written, the scheme is valid in the presence as well as in the absence of oxygen. Some of the reactions, e.g. the formation of pyruvic acid, have been shown to take place both in digests and in the living tissues. Even when the latter has been shown it does not amount to a demonstration that our reactions are necessarily 'the mechanism of respiration'. Such a demonstration is at present impossible to come by, and we must be satisfied with the knowledge that these are possible and even probable means by which the plant may utilize its sugars. That they are the only means is not regarded as proven.

The reasons which lead us to presume the existence of the various reactions listed above are briefly recapitulated in the following paragraphs:

H_3PO_4 + *adenylic acid* ⟶ *adenyl phosphate*. Controlled acid hydrolysis of freshly prepared extracts with trichloracetic acid releases small quantities of

[1] Named from their relatively high resistance to artificial acid hydrolysis; not from their biological properties.

inorganic phosphate. The relations are such as to suggest its liberation from a labile ester such as adenyl pyrophosphate (Arney, 1939). The addition of adenylic acid to barley saps containing sugars and free inorganic phosphate, brought about the formation of pyruvic acid (James *et al.* 1941) and free CO_2 (p. 268, present paper), to be expected. It is also worth recalling that, during mild starvation when the sugars and resistant esters (probably hexosediphosphate, in the main) were being used up, the labile ester was found to be still present, and even increasing its amount (Arney, 1939). This behaviour suggests a carrier rather than a plastic substance. We have not yet attempted the isolation of adenyl pyrophosphate or similar compounds from barley, and therefore prefer the indefinite name adenyl phosphate for the presumed adenylic ester.

The sugars consumed. Direct analysis has shown that sucrose is the mainstay of barley glycolysis, both in young seedlings (James, 1940) and in mature leaves (Yemm, 1935). It may even be an intermediary in the breakdown of endospermic starch, being largely transferred to the embryo before consumption. Minor roles are also played by raffinose (James, 1940) and fructosans (Yemm, 1935). All these substrates yield pyranoses on hydrolysis, either glucopyranose or fructopyranose, or both, and it is a reasonable assumption that these are the sugars phosphorylated by the labile ester. They are known to be consumed by glycolysis and continuously regenerated until a late stage of starvation by hydrolysis of their oligosaccharides. The major product of their phosphorylation—hexosediphosphate—is also known to be formed.

It is a remarkable fact that all the substrates mentioned above, viz. sucrose, raffinose and the fructosans, are furanosides; and pyranoses and pyranosides such as maltose and the hemicelluloses are less readily consumed. At times when the furanosides are rapidly disappearing, the pyranoses and their derivatives may even accumulate (cf. W. O. James & A. L. James, 1940). There is also some evidence that barley digests which have been rendered incapable of breaking down pyranose may still be able to decompose sucrose, though this has not proved to be so invariable or so strongly marked as was at first supposed. This special lability of the compounds of fructofuranose, which is itself incapable of continuous existence in the free form, may indicate a preferential decomposition in glycolysis. Whether this merely implies an increased facility of phosphorylation or some other independent glycolytic process is at present quite uncertain.

Adenyl phosphate + pyranose ⟶ adenylic acid + hexosediphosphate. We have not yet studied this reaction in isolation, but are led to infer it on several grounds. Its product, hexosediphosphate, was likely to be the main component of the 'resistant esters' estimated by Arney, and subsequent work not yet published in detail has confirmed this supposition. The activity of hexosediphosphate in giving rise to later members of the series—pyruvic acid and CO_2—has now been shown at some length. The sugars examined, sucrose and glucose, will only produce the same results in barley saps when adenylic acid and phosphate are also present. It thus seems a fair inference that the adenylic acid phosphorylates glucose in our barley saps and tissues as it is already known to do in yeast.

Hexosediphosphate —→ phosphoglycerate. Phosphoglycerate has not yet been isolated from barley, but when added to barley preparations it gives rise to the same intermediate and final products as hexosediphosphate, viz. pyruvic acid and CO_2. Addition of $M/40$ NaF blocks these reactions when either hexosediphosphate or phosphoglycerate is the substrate added, thus affording evidence of the initial conversion of the hexosediphosphate to phosphoglycerate.

Phosphoglycerate —→ pyruvate. Pyruvic acid has been isolated and identified from barley tissue to which carboxylase poisons have been applied (G. M. James & W. O. James, 1940), and also from digests to which phosphoglycerate has been added (James *et al.* 1941). In the absence of a carboxylase poison, added phosphoglycerate led to additional formation of CO_2 (p. 270).

Pyruvic acid —→ CO_2 + *acetaldehyde.* The decomposition of pyruvic acid by barley preparations and live tissues with the formation of acetaldehyde and CO_2 was first shown by James & Norval (1938) and has been confirmed in the paper preceding this (Bunting & James, 1941). In the present paper we have also shown the breakdown to CO_2 of a number of substances previously shown to give pyruvic acid.

Our method in general has been to work backwards from pyruvic acid and CO_2. Every preceding substance given in the tabulation has now been shown to give rise to both of them, under the influence of barley systems. Similar demonstrations have also been obtained for living barley tissues. Pyruvic acid has been isolated from them, and CO_2 production related to their consumption of sucrose, raffinose, fructosans, gluco- and fructopyranose, 'hexosediphosphate' and pyruvic acid.

SUMMARY

1. Adenylic acid gave rise to an increased CO_2 production when added to barley saps containing sugars and free phosphate. The increase was considerable, and apparently was not limited by the capacity of the adenylic acid, but by that of the barley carboxylase present. The dried extracted residue of the barley was also effective in raising the CO_2 output of the sap; but 1 mg. adenylic acid had a greater effect than 200 mg. of dried residue.

2. Hexosediphosphate also increased the CO_2 output of barley saps and other barley preparations. Its effect was completely inhibited by $M/40$ NaF.

3. Phosphoglycerate increased the CO_2 output of the digests by about the same amount as hexosediphosphate.

4. These increases were all exceeded by the increase of CO_2 output after addition of pyruvic acid under similar conditions.

These facts are considered in relation to others previously recorded, and the outline mechanism of glycolysis which they suggest is schematized on p. 272.

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THE DEPRESSANT EFFECT OF CARBON DIOXIDE UPON PHOTOSYNTHESIS

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(With 8 figures in the text)

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Introduction

Some of the earliest studies in photosynthesis were concerned with the relation of the assimilatory activity of green plants to the carbon dioxide concentrations of their environments. Although these were made at a time when the importance of the interaction of factors determining the rate was not understood, it was established that with increasing concentration of carbon dioxide the rate of assimilation rose to a maximum and then declined. The focus of interest was the establishment of 'optima'. The optimal concentration varied for different species. While Godlewski (1873), Kreusler (1885) and others showed that the concentration above which assimilatory activity declined was about 7–10 %, other estimates were as high as 20 %.

It has generally been assumed that a concentration of carbon dioxide of from 4 to 5 % is distinctly lower than that causing a decline in activity. Willstätter & Stoll (1918) performed the great majority of their large range of experiments in air streams containing concentrations of 4–6 %, and many modern workers have followed their methods.

Apart from the known effects of very high concentrations of carbon dioxide upon stomatal aperture, the possible causes of the decline of assimilation rate when the 'optimal' concentration of carbon dioxide is exceeded have been incompletely understood and are usually referred to in vague terms. Thus Blackman (1905) says: 'Ultimately, if the supply of carbon dioxide in the air current be increased up to 30, 50, 70 %, the carbon dioxide will have a general depressing effect on the whole vitality, and before suspension of all function a diminution of assimilation undoubtedly occurs. This is, however, quite a separate process.' Both Stiles (1925,

p. 89) and Spoehr (1926, p. 129) refer to the 'narcotic' effect of carbon dioxide upon assimilation.

Relative to this problem the results of some determinations of temperature coefficients of assimilation rate and drifts of assimilation rates at different temperatures are presented as providing further information about this depressant effect of carbon dioxide. Further work, for which opportunity was not available, would be necessary completely to define all the aspects of this effect.

Since the experiments upon which this report is based were performed, Livingstone & Franck (1940) have described experiments in which the depression of assimilation rate in atmospheres containing up to 20 % carbon dioxide was measured. They describe the depression as being qualitatively similar to that caused by cyanide.

Apparatus and material

The experiments reported in this paper were all performed with Blackman's palladium black apparatus which measures the oxygen output of assimilation (Briggs, 1920). It is necessary to refer to one feature of the apparatus not previously described.

The material is illuminated in a small chamber in an atmosphere of hydrogen to which is added an appropriate amount of carbon dioxide. The chamber forms part of a closed system, and the gases are continuously circulated over palladium black which catalyses the formation of water from the hydrogen and any oxygen produced in assimilation. The volume of the synthetic water is negligible, and the resulting decrease in volume is measured by a burette in circuit with the rest of the apparatus. At intervals more carbon dioxide is added to replace that gradually used in assimilation. There is thus a changing concentration of carbon dioxide and, since palladium adsorbs this gas in addition to hydrogen, it is necessary to determine whether the gas-palladium equilibrium varies. If so the volume changes will not entirely represent the course of assimilation.

It is only the net volume changes that are of importance when the palladium is brought into equilibrium with varying amounts of carbon dioxide and hydrogen. We are not concerned with the manner in which the adsorption of carbon dioxide and displacement of hydrogen when carbon dioxide is added to hydrogen-saturated palladium, or the adsorption of hydrogen and liberation of carbon dioxide when carbon dioxide is removed from the doubly saturated system, contribute to the net changes.

It has been shown that when the palladium black is maintained in a sufficiently dry state, addition of carbon dioxide in excess of that required to produce a concentration of 2·5 % results in an increase in volume of the gases practically equal to the volume of carbon dioxide added. In all the experiments this state of affairs was maintained, and hence changes in volume down to 2·5 % carbon dioxide give an accurate measure of assimilation.

Moreover, equilibrium is attained rapidly, and thus only short intervals are required after additions or removals of carbon dioxide before it is possible to proceed with assimilation measurements.

The illumination was provided by a gas-filled projection lamp with a small, uniplanar filament. The numerical values of intensities, given in lux, are approximate only, since it has been assumed that the illumination per watt is equivalent to 2 c.p. and that the energy is uniformly distributed over a sphere. Furthermore, in calculating intensities it has been assumed that the inverse square law holds for the distribution of light from the lamp. Over the range of intensities considered this assumption has been found by measurements with a photoelectric cell to approximate very closely to the truth. Thus while the absolute stated values of intensities may differ from the true values, the ratios these stated values bear to one another are correct.

Cotyledons of *Ricinus communis* L. and the leaves of *Ligustrum vulgare* L. have been used as material.

EXPERIMENTAL

Temperature coefficients

The following observations have contributed to the derivation of the temperature coefficients (Q_{10}). The initial rate of assimilation at the higher temperature was always established by at least two readings, and the temperature was then lowered. Frequently there were more than two readings at the lower temperature. After a period at the lower temperature there was always a return to the higher temperature. In this way any drift in assimilation has been established, and the two values from which the Q_{10} is calculated are the assimilation at the lower temperature for the middle period, and the assimilation at the higher temperature to correspond in time with this, obtained by interpolation on the time curve.[1]

In these experiments the intensity of illumination was 36,000 lux, which for this material at 16° C. had been previously shown to be of such magnitude that increasing the intensity had a negligible effect on the rate of assimilation apart from the indirect effect via increase of leaf temperature. The Q_{10}'s are thus those of the chemical stage of the process.[2]

Table 1 summarizes the results. The Q_{10}'s are those of apparent assimilation.

These values are much higher than those usually recorded.[3] The following values for Q_{10}'s of real assimilation (obtained by using dark respiration values), at intensities of illumination and carbon dioxide concentrations which make it clear they are for the chemical stage of the process have been found: 4·3 for *Chlorella pyrenoidosa*, 5·4–10° C. (Warburg, 1919); 4·6 for *Chlorella vulgaris*, 4–14° C. (Emerson, 1929); 1·87 and 2·15 for *Hormidium flaccidium*, 12–20° C. (van den Honert, 1930 and van der Paauw, 1932, respectively). For angiospermous leaves various

[1] It is realized that the interpolated value for the higher temperature may not be the same as that which would be measured if an observation could be made with an instantaneous change of temperature. The procedure adopted seems the most justifiable with our present knowledge, and is to be preferred to neglecting drifts completely.

[2] Later it will become evident that our Q_{10}'s, though independent of light, are dependent on carbon dioxide concentration.

[3] Blank determinations have been made which show that volume changes of the apparatus, or disturbance of the gas-palladium equilibrium as the temperature is changed, have negligible effect on the calculated Q_{10}.

workers have found values of 1·5–2·5 (Blackman & Matthaei, 1905; Willstätter & Stoll, 1918; Matthaei, 1905). Lundegårdh (1924) alone appears to have values approaching the present ones—5·0 for *Cucumus sativus*, 5·6 for *Solanum tuberosum* and 6·4 for *Solanum Lycopersicum*, 5–15° C.

If we assume a respiration rate of 3 mg. of carbon dioxide per hour per g. dry weight (and this is a reliable average figure) for *Ricinus* cotyledons at 15 or 16° C., and a Q_{10} of 2·0 for the respiration (this is equivalent to a respiratory value one-tenth that of the apparent assimilation at the higher temperatures, and one-half to one-third that at the lower temperatures), our Q_{10}'s for the range 6–16° C. are reduced only to 7·6, and we should have to assume an impossibly large respiration rate to reduce our values to the level of those usually found even for the algal systems.

Table 1

Description of material	Range of temp. °C.	Q_{10}
Ricinus cotyledons:		
m, fully green, 43 days*	15·00– 5·68	10·06
α_1, yellow-green to green:		
5 days	15·00– 5·76	9·65
8 days	15·00– 6·23	7·29
α_3, yellow, 15 days	15·00– 6·00	6·65
β_2, fully green:		
9 days	16·00– 6·00	8·67
12 days	16·00–11·00	3·16
δ_6, fully green, 8 days	16·00– 6·00	10·0
	16·00–11·00	5·35
	11·00– 6·00	19·1
Ligustrum leaves:		
Mature leaf	16·00– 6·00	4·3
		3·85
Mature leaf	16·00– 6·00	3·8

Many other records of assimilation at 16 and 6° C., but not for the same leaves, suggest a value of about 4 for the Q_{10} of *Ligustrum*.

* Age in days is measured from appearance above ground, which occurred on the fifth or sixth day after sowing.

Relation between assimilation rate and concentration of carbon dioxide

(*a*) *Experiments with* Ligustrum.

The data to be presented in this section fall into two classes: those derived from experiments of long duration (up to 14 hr.) in which the material has been subjected to a wide range of carbon dioxide concentrations, and those from experiments in which a smaller range of carbon dioxide has been employed. This limited range, however, has been repeated several times in the course of one experiment. Data of the first type were obtained from experiments performed specifically to obtain information on the present problem, those of the second were obtained by analysis of records not collected with this end in view.

In the course of experiments with *Ligustrum* at low temperature, 6° C., difficulty was experienced in obtaining constant or steadily drifting assimilation rates. Such difficulty had not been experienced at higher temperatures. In these experiments the initial concentration of carbon dioxide was about 4–5 % by volume, and when this had been reduced to 2·5–3 % more was added to restore the concentration to

approximately its original value. An inspection of the records of such experiments showed that the fluctuations in assimilation rate were related to these additions of carbon dioxide.

Fig. 1 presents graphically[1] the results of an experiment using two leaves at 6° C., and intensity of illumination = 36,000 lux. From *A* to *B*, as the concentration of carbon dioxide was reduced by assimilation, the rate steadily rose. At *B* carbon dioxide approximately equal in amount to that assimilated in the interval *A–B* was added, and after a small interval for equilibration with the palladium black, the rate was found to be depressed to *C*. Essentially the same course was followed

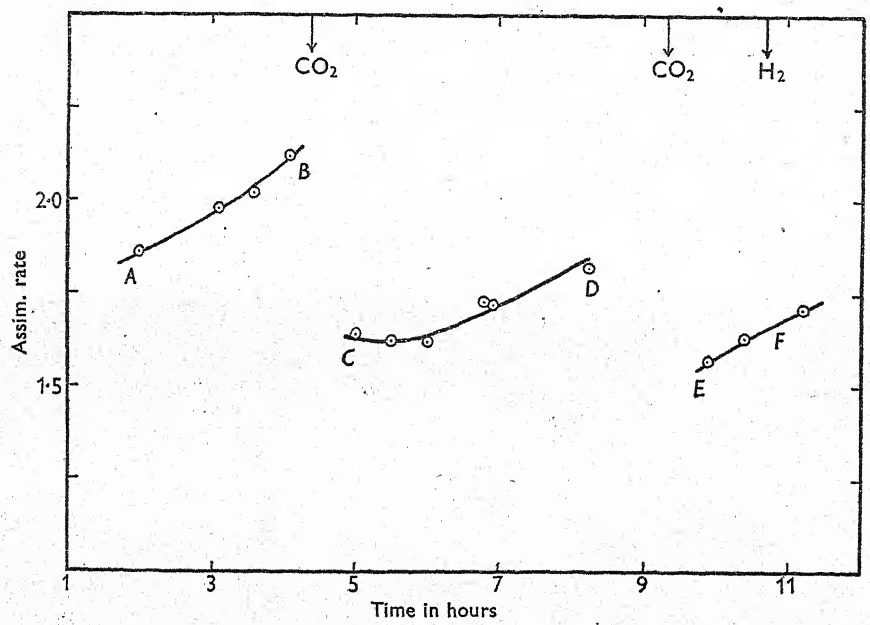

Fig. 1. Drift of assimilation rate of *Ligustrum* in arbitrary units at 6° C. and 36,000 lux.

when at *D* carbon dioxide was again added. At point *F* hydrogen was added. The amount was too small to have a marked dilution effect, and the drift of the rate remained undisturbed.

Commercial carbon dioxide had been used in the earlier experiments referred to above, and there is the possibility that this depressant effect was due to traces of some impurity which was progressively removed by the material or some other portion of the system. This explanation is rendered improbable by the fact that in this experiment, while commercial carbon dioxide was originally present in the apparatus, the additions at *B* and *D* were of purified carbon dioxide from a Kipp and commercial carbon dioxide respectively. The phenomena observed in each case were similar and may be attributed to the carbon dioxide.

[1] Since we are here primarily interested in relative rather than absolute magnitudes, all the results of this section are given graphically, and since respiration was a small fraction ($< 1/20$) of the apparent assimilation, this latter has been used.

It is not intended to imply that the whole of the changes of rate with time are attributable to change of carbon dioxide concentration alone. It is well known that there also occur drifts which are more to be correlated with age or length of exposure in the apparatus. For convenience these drifts, due to changes within the assimilating material, will be referred to as 'time' drifts. The nature of these will not be further discussed at present. In the experiment under discussion, in so far as the additions of carbon dioxide at *B* and *D* restored the original concentration, the points *A*, *C*, *E* indicate a downward 'time' drift, and hence the rise of assimilation rate due to the decrease in the carbon dioxide concentration is greater than is at first apparent.

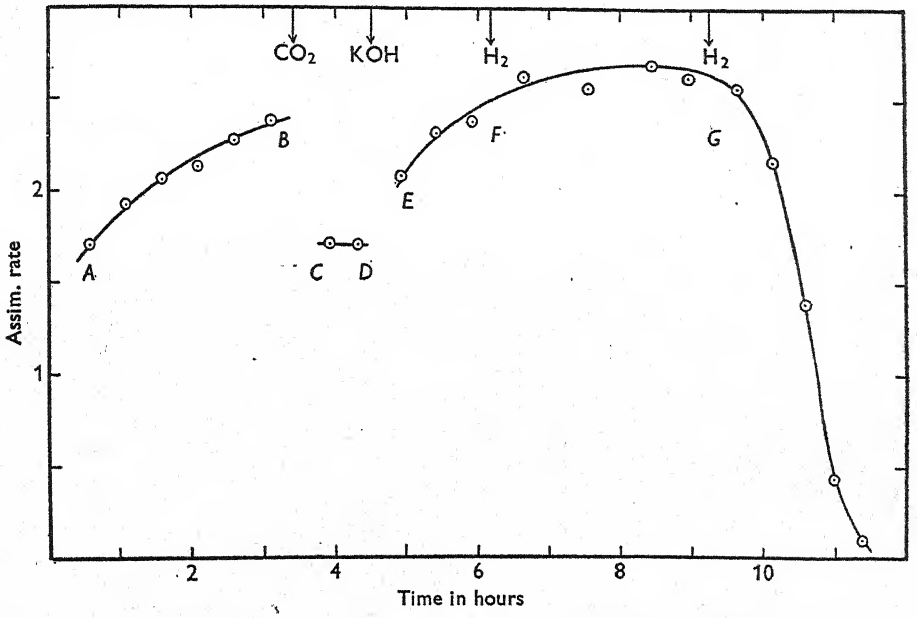

Fig. 2. Drift of assimilation rate of *Ligustrum* in arbitrary units at 6° C. and 36,000 lux.

The next step was to determine whether at these low temperatures there is a range of carbon dioxide concentrations over which assimilation rate is sensibly constant. The existence of such a range of concentrations at higher temperatures has already been established.

Fig. 2 is the record of results from two leaves at 6° C. and 36,000 lux. The course of the experiment was as follows: *A* was the first reading, at *B* more carbon dioxide equivalent to that assimilated in the interval *A*–*B* was added, at *D* the concentration of carbon dioxide was reduced by circulating the gas mixture through potash. At *F* and *G* hydrogen was added in a small amount insufficient to affect appreciably the carbon dioxide concentration. These results leave little doubt as to the presence of a depressant effect of carbon dioxide at low temperatures; the sharp rise to *E* following removal of some of the carbon dioxide by the potash and the subsequent steady rise would be difficult to explain on any other basis. The

decline in rate to a value approaching zero is an indication that the concentration had been reduced to the region where assimilation rate is dependent upon the concentration of carbon dioxide as a *reactant* in the photosynthetic process.

By assuming that the assimilatory quotient $O_2/CO_2 = 1$, and that there is no carbon dioxide remaining at the end of the experiment, it is possible to calculate the percentage of carbon dioxide over the various time intervals from the observed changes of volume of the gas in the apparatus.[1]

Such data for the experiment quoted above are presented in Fig. 3. Since the carbon dioxide changed appreciably over the intervals, the average assimilation

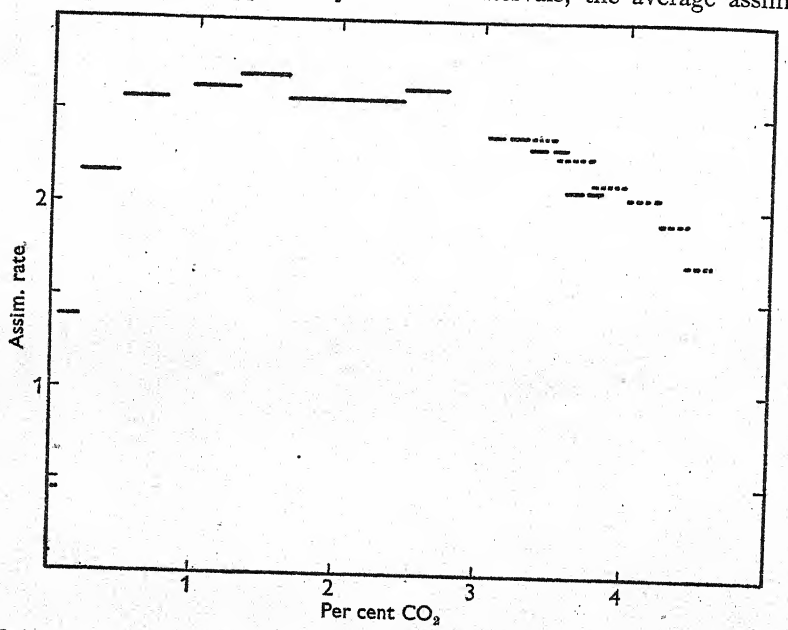

Fig. 3. Relation between assimilation rate and carbon dioxide concentration for *Ligustrum* at 6° C. and 36,000 lux. Dotted line for initial state, broken line after addition of carbon dioxide, full line after addition of hydrogen.

rates have been plotted against the limits of the carbon dioxide concentrations at the beginning and end of the periods. Where a given concentration of carbon dioxide has occurred more than once in the experiment, it will be seen that the points are close together, and it seems justifiable to conclude in this case that 'time' drifts were not of great magnitude and the changing rate was due mainly to changing carbon dioxide concentration. Here, concentrations above 2·5 % have depressed the rate. Below this value assimilation has remained steady till about 0·75 % carbon dioxide, at which point the concentration becomes 'limiting'.

[1] There are reasons why the use of such an apparatus does not give strictly accurate results for carbon dioxide determinations. Towards the end there will be some carbon dioxide released from the palladium black for which no allowance is made, and this is at a stage where such discrepancies will make the greatest percentage difference. Also any traces of oxygen that are introduced during addition of carbon dioxide or hydrogen, and during circulation through potash, become magnified three times and are calculated as carbon dioxide. For such reasons it is not desired to stress the actual values recorded, but rather the general form of the relationships.

In order to facilitate comparison with similar experiments a smooth curve has been drawn through these lines, and values at regular intervals of carbon dioxide concentration obtained by interpolation. Since the same range of carbon dioxide concentrations has not been employed in all experiments, it has been necessary to select one concentration common to them all and express the rates at the other concentrations as percentages of this. The concentration selected as standard has been 3·5 %, the lowest value common to all experiments.

The data for a number of experiments treated as above are collected in Fig. 4. In this and similar succeeding figures the rates on the first occasion when the

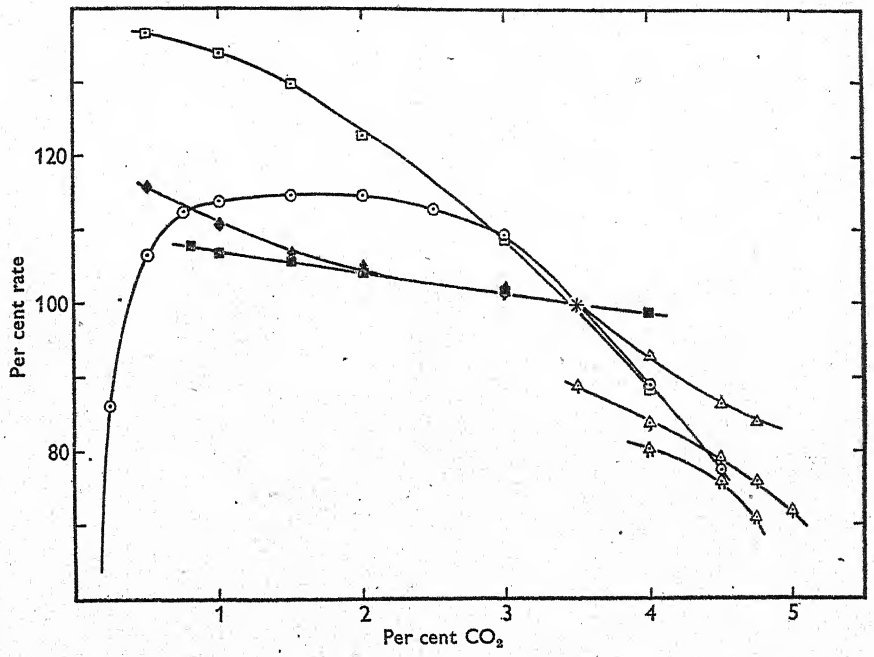

Fig. 4. Relation between assimilation rate (expressed as percentage of that at 3·5 % carbon dioxide) and concentration of carbon dioxide for *Ligustrum*. Temp. = 6° C. Open symbols 36,000 lux, solid 2000 lux.

carbon dioxide concentration reached 3·5 % are taken as 100, and values obtained during periods following on addition of carbon dioxide are denoted by an appropriate number of strokes on the main symbol.

Considering first the experiments at high intensities of illumination it is evident that as the concentration falls below 5 % there is a marked rise in assimilation rate (60 % or more) till about 2·5 % is reached. There is one curve continually rising above 2·5 %. This may well be the expression of an upward 'time' drift.

There are other experiments for which the exact figures are not available. In these the carbon dioxide was allowed to fall from approximately 4·5 % to approximately 3 %, and coincident with this the assimilation rates rose 20–30 %. This agrees with the rise over the corresponding portions of the more complete curves.

No experiment at low temperature and high intensity of illumination failed to show this rise in rate.

This is markedly different from the state of affairs when the intensity of illumination is reduced to a low value or the temperature is raised to 16° C. In Fig. 4 are also records from two experiments at 6° C. and intensity of illumination = 2000 lux. While independent experiments have shown that this intensity is such that the rate of assimilation is very nearly proportional to the intensity of illumination at 16° C., the rate will probably not be so close to proportionality at 6° C., but how far removed we cannot say. At this low intensity of illumination the increases in rates of assimilation over the range 4–0·5 % carbon dioxide are 8 and 15 % respectively—of the order of one-fifth of the relative magnitudes observed at high intensities of illumination.

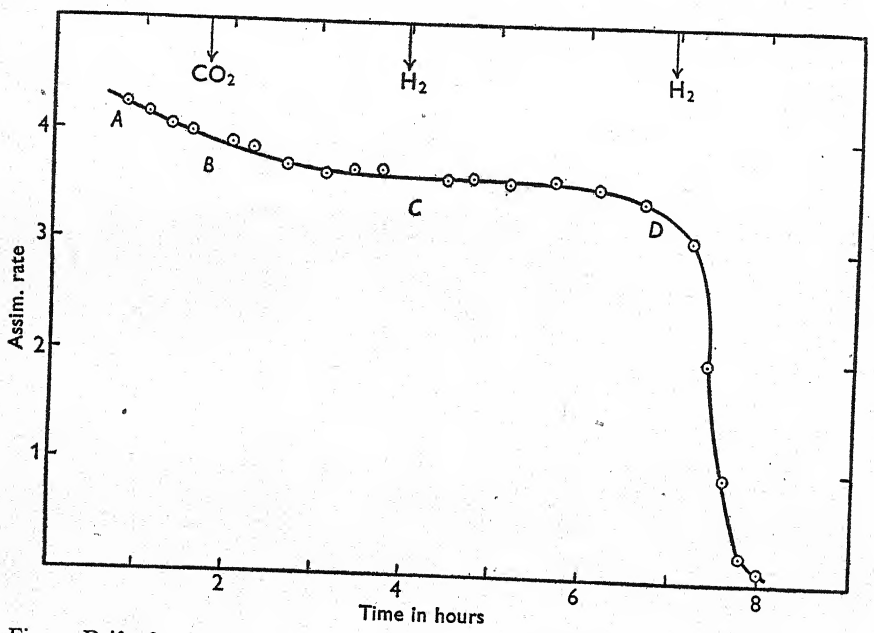

Fig. 5. Drift of assimilation rate of *Ligustrum* in arbitrary units at 16° C. and 36,000 lux.

That these differences between the increases at low and high illumination are due wholly to the nature of the material or superimposed 'time' drifts is discounted by the following facts. In one of these experiments the carbon dioxide concentration fell from a high to a lower value during the day, in the other it started at a low value and reached a higher one after additions of carbon dioxide. Further, in one experiment the material was exposed alternately to the high and low intensities of illumination throughout the day, and this was the material which exhibited the very marked upward 'time' drift referred to above.

During many experiments with *Ligustrum* at 16° C. there has been no suggestion of increasing rates due to decreasing concentrations of carbon dioxide. Fig. 5 presents the results of a typical experiment. The general form of the drift is

seen to be a decline, marked at first, gradually merging into a steady state. The addition of carbon dioxide at *B*, and the dilution by adding a relatively large amount of hydrogen at *C*, have done nothing to disturb this drift. Fig. 5 is in complete contrast to Fig. 2.

The carbon dioxide relationships for this and other experiments at high temperature are presented in Fig. 6. There is no evidence of a depressant effect of carbon dioxide superimposed upon 'time' drifts. This is true for many other experiments for which the exact figures are not available.

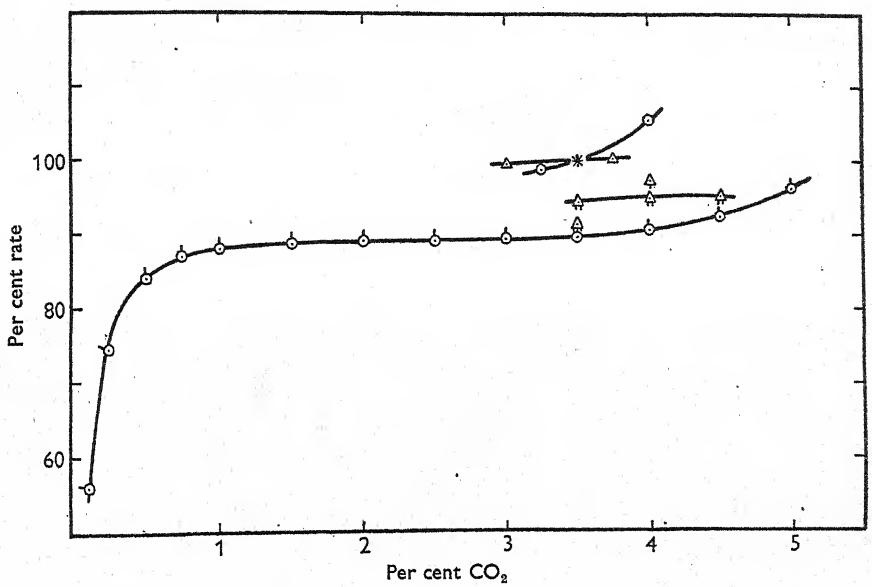

Fig. 6. Relation between assimilation rate (expressed as percentage of that at 3·5 % carbon dioxide) and concentration of carbon dioxide for *Ligustrum*. Temp. 16° C. Circles 36,000 lux and triangles 19,700 lux.

(*b*) *Experiments with* Ricinus.

Fig. 7 presents results from two experiments with *Ricinus* cotyledons at 6° C. and 36,000 lux, which, taken together, offer good evidence that a similar carbon dioxide depression at low temperatures occurs here also. Approximately the same ranges of carbon dioxide concentrations were employed here as with *Ligustrum*. The curves are not as regular as those of *Ligustrum*, and it is possible that long exposure to low temperatures is harmful to the cotyledons of *Ricinus communis* (which is a subtropical species). After 9 or 10 hr. in the apparatus at 6° C. the cotyledons were limp and did not appear normal, in contrast to the leaves of *Ligustrum* at a similar temperature, and *Ricinus* cotyledons at temperatures of 16° C. and above. These data have not been expressed quantitatively in terms of carbon dioxide concentrations.

Data for typical experiments at higher temperatures are given in Fig. 8, and it is at once clear that there is no general rule of behaviour. Consideration of records

of other experiments, bearing in mind the possibility of 'time' drifts, leads to the same conclusion, viz. that in some cases a slight depression has occurred, and in others none at all.

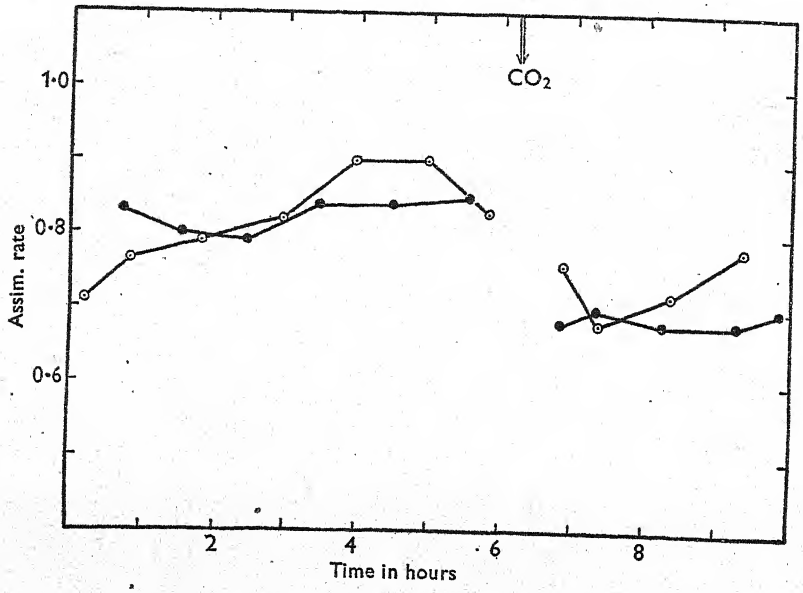

Fig. 7. Drift of assimilation rate in arbitrary units for two *Ricinus* cotyledons at 6° C. and 36,000 lux.

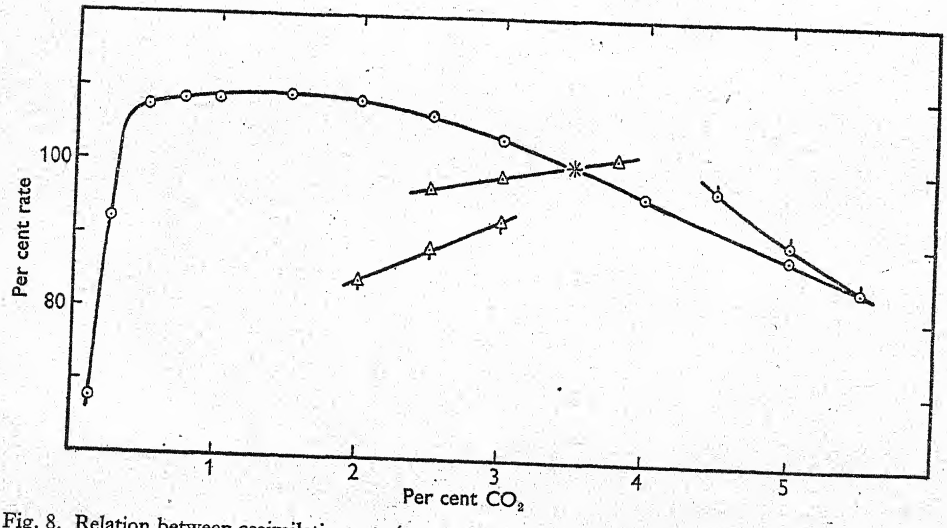

Fig. 8. Relation between assimilation rate (expressed as percentage of that at 3·5 % carbon dioxide) and concentration of carbon dioxide for *Ricinus*. Temp. = 16° C. Circles 36,000 lux and triangles 19,700 lux.

Discussion

The results of the preceding sections may be summarized as follows. The Q_{10} (6–16° C.) of the assimilation rate of *Ricinus* cotyledons is greater than that of *Ligustrum* leaves over the same temperature range, and is also greater than those usually found for other assimilating systems. At low temperatures with high intensities of illumination the rates of assimilation of *Ligustrum* leaves and *Ricinus* cotyledons are depressed when the concentration of carbon dioxide rises above a certain value which is probably 2–2·5 %. With decrease in intensity of illumination the depressions are smaller, while raising the temperature to 16° C. practically removes the depression for *Ligustrum* leaves in concentrations of carbon dioxide up to 5 %; but not so completely for *Ricinus* cotyledons.

These results are of interest in connexion with various kinetic formulations of the photosynthetic system that have been attempted. Emerson (1936, 1937) has criticized such formulations on the grounds of their speculative nature, and claims they can exert no directive influence on our attempts to investigate the process further. Such is not the case as Briggs (1935) has shown. Moreover, our knowledge of the photosynthetic system would advance but slowly if we were to adopt the only other alternative, viz. to identify as chemical entities all the intermediate products between carbon dioxide and carbohydrate before attempting to correlate the mass of data that exists. The consideration of such schemata by no means implies a complete finality for them; they enable precise thinking in a complex subject and may be modified as fresh data are obtained.

Such a formulation by Briggs (1933, 1935) seems best to fit the wide range of facts available. According to this schema carbon dioxide combines with a substance S in the cell to form a compound S_c which after activation, directly or indirectly, by light energy is broken down with the aid of a catalyst B to the products of photosynthesis. The activated S_c and the catalyst B form a complex X. To explain the depressant effect of a substance such as hydrogen cyanide, which depresses the rate of photosynthesis more at high intensities of illumination than at low, it is assumed that hydrogen cyanide decreases the amount of available B by combining with it. The above findings concerning the depressant effect of carbon dioxide at high concentrations can be explained by assuming that carbon dioxide acts in a manner similar to hydrocyanic acid.

If the dissociation constant of the compound of B with carbon dioxide increases with rise of temperature, the reduction of depressant effect with increase of temperature is explained. At temperatures about 16° C. this complex of B and carbon dioxide is practically all dissociated, while at lower temperature there are increasing amounts accompanied by increasing depression of rate. Moreover, the operation of such an inhibitor would account for the high Q_{10}'s observed. (Assuming there to be no depression at 16° C. for *Ligustrum*, the depressions observed at 6° C. would increase the Q_{10}'s 1·5–2 times. Since in some cases there is a residual depression at 16° C. with *Ricinus*, greater depressions than those observed for *Ligustrum* would be required to bring about similar increases of Q_{10}'s by the operation of this factor.)

The results for the *Ricinus* cotyledons β_2 and δ_6 indicate that the Q_{10} of photosynthesis falls off very rapidly with rise of temperature. This is in agreement with the results of other workers, though there the fall is much smaller than in the present experiments. The value of α_3 resembles the findings of Willstätter & Stoll (1918) that yellow leaves have smaller Q_{10}'s than green leaves. According to his schema Briggs has offered explanations for both these facts. The explanation of the former is that the dissociation constant of X, the compound of B and activated S_c, increases with temperature and hence there is less X as the temperature rises. The explanation for the second is that in the leaves with the higher Q_{10}'s there is sufficient S to keep nearly the whole of B in the X form at the higher temperatures, while in the others the ratio of S to B is less, and so the percentage fall of X with rise of temperature is greater. Similarly, if the amount of effective B substance is reduced by excess carbon dioxide (in a manner analogous to the action of hydrogen cyanide), the reduction in assimilation rate by combination of B with carbon dioxide will be greater with high intensities of illumination when most of the S_c is in the activated form than with low intensities when only a small portion of the S_c is activated. This is in agreement with the observed depressions at high and low intensities of illumination.

The different behaviour of the *Ligustrum* leaves and *Ricinus* cotyledons at 16° C. may be explained if we assume that the ratio S/B varies with species and among individuals. We should then regard *Ligustrum* leaves as having a smaller S/B ratio than most of the cotyledons of *Ricinus* used. At light saturation with *Ligustrum* inactivation of some of the B substance by combination with carbon dioxide will inappreciably alter the amount of S in the X form and hence cause a relatively small depression in the rate of assimilation. *Ricinus*, on the other hand, with, in most cases, a larger amount of S relative to B will show a greater depression. Other cases with a smaller S/B ratio behave like *Ligustrum* leaves.

Turning to the consideration of the Q_{10} in the presence of high concentrations of carbon dioxide, let us consider the case where the depression due to carbon dioxide is negligible at the higher temperature of 16° C. The depression at 6° C. will be greater the higher ratio of S/B. Hence, on account of the carbon dioxide factor, the Q_{10} will be higher the greater this ratio. As pointed out before the decrease of the amount of B in the X form with increase in temperature will be less the greater the ratio of S/B. Hence both factors work in the same direction, and the marked difference in the Q_{10} of *Ricinus* (8–10, for a rise of 10° C.) and *Ligustrum* (4) can be attributed, in part at least, to a higher ratio of S/B in the former.

All these considerations lead to the conclusion that carbon dioxide, the chief reactant in photosynthesis, also acts as an inhibitor of the process, preventing the maximum rate otherwise possible from being ever attained, and under the conditions stated above the reduction becomes appreciable.

SUMMARY

High temperature coefficients for the rate of apparent assimilation are found for *Ricinus* cotyledons (8–10) and *Ligustrum* leaves (4) over the temperature range 6–16° C.

Concentrations of carbon dioxide above 2–2·5 % depress the rate of assimilation of leaves of *Ligustrum* at 6° C. The percentage depression is greater at high intensities of illumination than at low. No such depression occurs in concentrations up to 5 % at 16° C.

A similar depression at 6° C. occurs with the cotyledons of *Ricinus*, and sometimes also at 16° C.

These facts are interpreted on the basis of carbon dioxide itself acting as an inhibitor of the hydrogen cyanide type by combining with the catalyst *B* which, in the dark or chemical reaction, facilitates the breakdown of a light-activated compound of carbon dioxide with a substance *S*. This explains the greater percentage depression at high intensities of illumination. In order to explain the more marked depression at low temperatures it is assumed that the dissociation constant of the compound of carbon dioxide with the substance *B* increases with rise of temperature. This also partially accounts for the high temperature coefficients observed.

The different behaviour of the two types of material may be explained by assuming different ratios of the substance *B* to the substance *S*.

The work was performed during the tenure, from 1934 to 1937, of a Scholarship of the Royal Commission for the Exhibition of 1851.

The author is greatly indebted to Mr G. E. Briggs, F.R.S., for advice and encouragement during the course of the investigation.

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ABSORPTION, TRANSLOCATION AND ULTIMATE FATE OF LITHIUM IN THE WHEAT PLANT

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Introduction

During the course of experiments with lithium on wheat and its susceptibility to mildew (*Erysiphe graminis* DC.) and brown rust (*Puccinia triticina* Eriks.) (Kent, 1941) it became necessary to collect more data concerning the movement of lithium within the wheat plant, particularly as to whether lithium deposited in the older leaves could be re-exported to the younger leaves and influence their susceptibility and growth. Accordingly, a preliminary experiment was set up to investigate the rate of absorption of lithium by young wheat plants and its distribution between the first and second leaves; this was followed by a main experiment designed to give information regarding the movement and redistribution of lithium in plants following their transplantation from lithiated to untreated soil. By the transplantation technique it was hoped to interrupt the lithium supply before the first leaf was fully developed and to follow, by sampling, the fate of the lithium in that and the younger leaves.

Materials and methods

Wheat plants of the variety 'vulgare p.p.' were grown in a greenhouse from seed sown at the rate of about eight seeds per 4 in. pot. The lithium treatment, applied when the first leaves were well developed but before the second leaves had expanded, consisted of the application of 10 c.c. of $2\frac{1}{2}$ % lithium chloride solution to the surface of the soil in each pot (making 18·35 mg.-equiv. Li per l. soil); the control plants received no lithium. Some of the lithium-treated plants were transplanted at the appropriate time by removing them carefully from the soil, washing the roots quickly in water and setting them again in fresh untreated soil. For analysis the plants were separated into first, second, etc., leaves and roots. 'Leaf' included both lamina and leaf sheath (the latter extending down to the base of the coleoptile); the remains of the seed were included with the roots. The parts were analysed for lithium spectrographically by a modification of the Ramage flame method, details of which have been published elsewhere (Kent, 1940).

Experimental results

Preliminary experiment

A set of plants of uniform age was divided into three groups: untreated (C), lithiated and transplanted (LT) and lithiated only (L). Group LT was transplanted to untreated soil 3 days after lithium treatment. All the groups were taken up for

analysis 27 days after groups L and LT had been treated with lithium. The end point of 27 days was chosen as the time when the first leaf had withered but the second was still green. In this experiment only the first and second leaves were collected. The dry and ash weight yields of these parts, together with the results of the analysis for lithium, are given in Table 1.

Table 1. *Weight and lithium analysis results of lithiated and control wheat plants (first and second leaves only)*

Treatment group	Leaf	Dry wt. per plant mg.	Ash wt. per plant mg.	Lithium conc. mg./100 g. dry	Lithium content per plant μg.
C, control	1st	6·26	1·31	7·8	0·49
	2nd	9·71	1·77	6·2	0·61
LT, lithiated and transplanted	1st	6·83	1·66	79·0	5·37
	2nd	9·77	1·94	35·5	3·47
L, lithiated only	1st	6·55	1·65	378·0	24·74
	2nd	8·93	1·93	411·0	36·77

The untreated plants contained a small quantity of lithium derived from the untreated soil. By subtracting this from the totals for the other two groups the increase in the amount of lithium absorbed, due to lithium treatment, can be deduced. This is termed the 'corrected lithium increment'. From these figures the rate of absorption per day and the percentage uptake were worked out, and these results are shown in Table 2.

Table 2. *Rate of absorption and percentage uptake of lithium by lithium-treated wheat plants (first and second leaves only)*

Treatment group	(*a*) No. of plants	(*b*) Days	(*c*) Lithium application per group mg.	(*d*) Corrected lithium increment per group mg.	(*e*) Corrected increment as percentage of amount supplied	(*f*) Corrected lithium increment per plant per day μg.
L, lithiated only	31	0–27	206	1·873	0·91	2·24
LT, lithiated and transplanted	35	0– 3	206	0·271	0·13	2·58
L, lithiated only by difference	31	4–27	206	1·633	0·79	2·20

Comparing the figure for the first 3 days (days 0–3: 2·58 μg. per plant per day) with that for the subsequent 24 days (days 4–27: 2·20 μg. per plant per day) it is seen that the rate of absorption by the first two leaves remained nearly constant with but little decrease after the first 3 days. The small figure for percentage uptake (0·91 %) during 27 days from lithiation shows that the lithium concentration in the soil—after making due allowance for unestimated lithium in the root and other leaves—was only slightly lower at 27 days than immediately after lithium application.

Discussion of preliminary experiment

Since some lithium was found in the second leaf sample of the transplanted group the result of the experiment is inconclusive as regards showing whether re-export of lithium from the first leaf has occurred. The lithium increment ($3{\cdot}47 - 0{\cdot}61 = 2{\cdot}86$) in the second leaf, 24 days after transplantation into fresh soil, may represent translocation from the first leaf or from the roots. In order to settle this point a larger experiment was carried out in which plants were sampled at the time of transplanting and analyses were made of roots as well as of foliage.

Main experiment

Introduction

The experiment comprised thirty-six 4 in. pots of wheat seedlings divided into twelve groups of three, each group having about twenty-two seedlings. There were four control and eight lithiated groups, and of the latter four were subsequently transplanted. At the start of the experiment all the L groups were lithiated. Groups C 3, C 10, C 27 and C 35 (controls) were sampled at 3, 10, 27 and 35 days respectively from the start of the experiment. Groups L 3, L 10, L 27 and L 35 (lithiated, not transplanted) were sampled similarly. Groups L 3 + C 24 and L 3 + C 32 (lithiated) were transplanted into untreated soil 3 days from the start and sampled 24 and 32 days respectively from transplanting, while the remaining two groups L 10 + C 17 and L 10 + C 25 were transplanted 10 days from the start and sampled 17 and 25 days later. The lithium treatment and process of transplanting into fresh untreated soil were precisely similar to those described in the preliminary experiment. In sampling, roots were removed by cutting at the base of the first leaf, the leaves then removed in order. The meristems were included with the youngest leaf. The analytical method was the same as that used in the preliminary experiment.

Weights and weight ratios

The fresh, dry and ash weights of all the various parts of the plant were recorded but it is sufficient to give only the fresh weights here (Table 3). Ten days from the start of the experiment (C 10 and L 10) the toxic effect of lithium treatment was beginning to show. There was little difference in the weight of the first leaf, which was to be expected since the lithium was applied when the first leaf was well developed. The effect was well marked on the second leaf and more prominent still on the third. The roots showed little difference after 10 days. The reduction in total fresh weight, due to lithium treatment, was considerable after 27 and 35 days, the effect being most prominently shown in the weight of the fourth leaf and root and negligible on the first leaf. The decrease in weight due to 3, 10 and 35 days lithium treatment (cf. the 35-day groups) is quite regular. A regular drift is observable in the ratio dry weight/fresh weight, which increases in passing from the first to the fourth leaves and is generally higher for the groups growing in treated soil

Table 3. *Fresh weight of parts of wheat seedlings (mg. per plant)*

Group	Sampling: days after lithiation	1st leaf	2nd leaf	3rd leaf	4th leaf	Root	Total
C 3	3	73	21	—	—	124	218
L 3	3	66	16	—	—	148	230
C 10	10	82	101	54	—	145	382
L 10	10	81	53	11	—	139	284
C 27	27	82	102	148	265	353	950
L 3+C 24	27	101	80	120	164	272	737
L 10+C 17	27	106	97	117	191	309	820
L 27	27	80	77	101	113	217	588
C 35	35	87	109	159	590	300	1245
L 3+C 32	35	92	85	122	425	200	925
L 10+C 25	35	78	69	86	270	234	737
L 35	35	58	61	84	178	136	517

for all or part of the time. The ash weight/fresh weight ratio and the ash weight/dry weight ratio, on the other hand, decrease from the first to the fourth leaves but are in general higher for the treated than for the untreated plants. These results are illustrated by the figures obtained with the 35-day groups (Table 4). At 10 and 27 days the drift is less pronounced but in the same direction. These results show that in the older leaves there is relatively more water and more ash than in the younger leaves and that in the treated plants there is relatively less water and more ash than in the untreated plants. Lithium treatment depresses net gain in water, dry weight and ash by the leaf during its growth, and the results of these experiments show that the depression is greatest for water and least for ash. It is not clear whether the resulting desiccation of the tissues is due to decreased water uptake or to increased transpiration.

Table 4. *Percentage weight ratios of parts of wheat seedlings*

Group	Leaves				
	1st	2nd	3rd	4th	Roots
	(*a*) Dry wt./Fresh wt. %				
C 35	10·6	13·0	14·2	14·9	13·7
L 3+C 32	11·4	13·9	15·1	14·3	13·2
L 10+C 25	13·0	15·8	17·2	15·2	11·4
L 35	13·9	15·6	16·6	17·3	16·1
	(*b*) Ash wt./Fresh wt. %				
C 35	2·77	3·02	2·88	2·05	2·59
L 3+C 32	3·56	3·35	3·09	2·32	3·00
L 10+C 25	3·52	3·35	3·23	2·87	3·15
L 35	4·05	3·30	2·75	2·27	3·52

Analysis for lithium

The lithium content in μg. per plant for the various parts of the wheat plant is given in Table 5. Inspecting the two series C 3, C 10, C 27, C 35 and L 3, L 10, L 27, L 35, it is seen that the lithium content continued to increase during the

whole period of growth. The controls, which received no lithium treatment, nevertheless contained at 35 days about one-ninth as much lithium as the treated plants. Inspecting the two transplanted series (L 3, L 3+C 24, L 3+C 32, and L 10, L 10+C 17, L 10+C 25) it is seen that after repotting into untreated soil the large quantity of lithium in the roots rapidly diminished; in the case of the 3-day treatment about 19 % of this decrease would be accounted for by translocation to the first leaf, about 8 % to the second, third and fourth leaves, leaving 73 % of that present in the roots, which was apparently returned to the soil.

Table 5. *Lithium content of various parts of the wheat seedling (μg. per plant)*

Treatment group	Leaves				Total tops	Roots	Total
	1st	2nd	3rd	4th			
C 3	0·03	0·01	—	—	0·04	0·26	0·30
C 10	0·23	0·26	0·06	—	0·55	0·78	1·33
C 27	1·89	2·74	2·53	2·61	9·77	6·21	15·98
C 35	2·16	4·29	3·18	5·55	15·18	5·19	20·37
L 3	7·80	0·84	—	—	8·64	29·38	38·02
L 10	18·35	5·62	0·63	—	24·62	22·50	47·12
L 27	33·77	25·29	27·60	7·28	93·94	41·00	134·94
L 35	45·23	34·15	30·36	19·45	129·19	54·60	183·79
L 3	7·80	0·84	—	—	8·64	29·38	38·02
L 3+C 24	13·14	2·02	0·91	0·32	16·39	1·06	17·45
L 3+C 32	13·53	2·12	0·53	0·99	17·17	1·74	18·91
L 10	18·35	5·62	0·63	—	24·62	22·50	47·12
L 10+C 17	18·00	15·29	2·37	1·15	36·81	4·50	41·31
L 10+C 25	17·81	11·96	2·57	1·59	33·93	4·70	38·63

Transplantation could not be accomplished without damaging the root hairs even using the utmost care when repotting. In consequence the absorbing power of the roots would be temporarily hindered until new root hairs had developed. Furthermore, lithium might be lost from the plant to the soil through the resulting wounds. Loss of lithium through broken root hairs in this way probably does not account for more than a small fraction of the total observed quantity of lithium lost to the soil.

In the case of the 10-day treatment much of the lithium that was in the roots at 3 days had already passed to the first and second leaves at the time of repotting (10 days). After 17 days in the untreated soil (group L 10+C 17) 80 % of the lithium in the roots had been lost, 43 % to the second leaf, 13 % to the third and fourth leaves and 27 % back to the soil. There was little variation in the lithium content of the first leaves of the three groups L 10, L 10+C 17 and L 10+C 25; following transplanting, there was no decrease in lithium content of the first leaf as there was in the case of the roots. Such fluctuations as were shown were well within the limits of experimental error. This suggested that although lithium in the root was mobile and could be translocated about the plant, the lithium in the first leaf was fixed and could not be moved away to other parts.

Loss of lithium from the roots to the soil after repotting (increase in the content of lithium in the leaves could not account for all the loss from the roots) suggests that the movement of lithium into and out of the plant is governed, in part at least, by the direction of the lithium gradient. The lithium content of the third and fourth leaves of the transplanted groups L 3+C 24 and L 3+C 32, L 10+C 17 and L 10+C 25 was actually less than that of the C 27 and the C 35 plants, growing respectively for the same periods of time in untreated soil, whereas that of the first leaves was considerably greater. This suggests that while the redistribution of lithium following transplantation is governed principally by the direction of the lithium gradient, this redistribution is hindered by immobility of lithium in the first leaf. A table of the fresh weight concentrations of lithium is appended (Table 6).

Table 6. *Lithium concentration in various parts of the wheat plant (mg./100 g. fresh)*

Treatment group	Leaves				Roots
	1st	2nd	3rd	4th	
L 3	11·9	5·4	—	—	19·9
L 3+C 24	13·05	2·55	0·75	0·19	0·39
L 3+C 32	14·60	2·48	0·43	0·23	0·87
L 10	22·6	10·5	5·6	—	16·1
L 10+C 17	16·92	15·86	2·03	0·60	1·41
L 10+C 25	22·88	17·42	2·97	0·59	2·02
C 3	0·04	0·04	—	—	0·21
C 10	0·28	0·26	0·10	—	0·53
C 27	2·32	2·72	1·70	0·99	1·76
C 35	2·49	3·93	2·00	0·94	1·73
L 27	42·2	32·8	28·6	6·4	18·9
L 35	71·2	51·5	33·0	12·1	36·5

The most striking figures in Table 6 are the root concentrations of the transplanted series, both of which show a considerable drop after repotting, e.g. from 19·9 to 0·39 mg./100 g., the latter figure being even smaller than that of roots of the same age but with no lithium treatment (group C 27 – 1·76 mg./100 g.).

Rate of lithium absorption

From the analysis of the lithium-treated groups an estimate of the amount of the supplied lithium that had been absorbed can be obtained by subtracting from each figure the value for the corresponding untreated groups, obtaining a 'corrected lithium increment' (see Table 7). The initial rate of absorption (days 0–3) was much larger (taking the plant as a whole) than that over the succeeding 32 days; after dropping to a very low figure in the period 4–10 days, the rate steadily rose. Taking the tops alone, there is no such fluctuation apparent, showing that the initial large rate of absorption only concerned lithium in the roots. The figures 2·9 and 1·9 μg. per plant (first and second leaves only) for the absorption rate per day over the periods 0–3 and 4–27 days respectively compare well with the figures 2·6 and 2·2 μg. obtained in the preliminary experiment (Table 2, col. *f*) under precisely

Table 7. *Rate of absorption of lithium by wheat plants*

Part of plant	Days of treatment	Lithium content per plant, μg.			Mean uptake per day (whole period) μg.	Lithium increment per plant per day μg.	
		Untreated	Treated	Treated (corrected)			
						Period	
Whole plant	3	0·30	38·02	37·72	12·57	0– 3	12·57
	10	1·33	47·12	45·79	4·60	4–10	1·15
	27	16·00	134·94	118·94	4·40	11–27	4·30
	35	20·36	183·79	163·43	4·69	28–35	5·56
						4–27	3·39
Tops only	3	0·04	8·64	8·6	2·9	0– 3	2·9
	10	0·55	24·62	24·07	2·4	4–10	2·21
	27	9·77	93·94	83·17	3·08	11–27	3·47
	35	15·18	129·19	114·01	3·26	28–35	3·85
						4–27	3·11
Leaves 1 and 2 only	3	0·04	8·64	8·60	2·87	0– 3	2·87
	10	0·49	23·99	23·50	2·35	—	—
	27	4·63	59·06	54·43	2·02	4–27	1·91
	35	6·45	79·38	72·93	2·08	—	—

comparable conditions of treatment, sampling, etc. The lithium treatment consisted of an application of 122 mg. of lithium per group. At the end of 27 days from lithiation the corrected percentage uptake was 2·15 %, and at the end of 35 days, 2·81 % of the lithium supplied. Whereas these figures 2·15 and 2·81 % relate to uptake by the whole plant, the figure 0·91 % for the uptake at the end of 27 days, given in the preliminary experiment (Table 2, col. *e*), relates to the first and second leaves only. The corresponding figure in the main experiment for first and second leaves only was found to be 1·03 %, showing close agreement. It was noticed in the lithiated (but not transplanted) series that lithium continued to accumulate in leaves whose dry weight had ceased to increase. This is illustrated in Table 8 by data for the first leaves.

Table 8. *Weight and lithium analysis results for the first leaves of wheat plants*

Treatment group	Weight per plant, mg.			Lithium content per plant μg.	Lithium concentration mg./100 g.	
	Fresh	Dry	Ash		Fresh	Ash
L 3	66·0	7·5	1·50	7·80	11·9	530
L 10	81·0	11·7	2·45	18·37	22·6	750
L 27	80·1	10·7	2·68	33·77	42·2	1260
L 35	58·0	8·1	2·57	45·23	71·2	1760

Discussion

Mason & Maskell (1931), in their experiments on the cotton plant, concluded that potassium, among other elements, was not fixed in the leaf but could be re-exported, passing through the phloem from the leaf towards the root where it

might retard the rate of salt absorption. On the other hand, there was no backward movement of calcium in the cotton plant; it was unable to move in the phloem. Nitrogen, phosphorus and potassium could be cut off early in the life of the plant without adverse effects, new leaves obtaining supplies by phloem transport or by re-export from older ones, but the need for calcium continued throughout life as the main supply of calcium to any tissue would arrive direct from the roots. Lithium resembles calcium in not being re-exported from the leaf and this may be due to failure of mobilization in the leaf or to the incapacity of lithium to move in the phloem. Birch-Hirschfeld (1920) found that the rate of movement of lithium through the phloem was small.

SUMMARY

The absorption and translocation of lithium by wheat seedlings under certain experimental conditions has been studied. The plants were analysed for lithium spectrographically by a modification of the Ramage flame method.

During the first few days after lithium treatment, young seedlings accumulated lithium in the roots; later the lithium was accumulated principally in the oldest leaves, where high concentrations were attained.

The experimental results suggest that lithium stored in the oldest leaves is immobile and cannot be translocated. In this respect lithium bears more resemblance to calcium than to the other alkali elements.

When the direction of the lithium gradient between the soil and the plant was reversed (by repotting lithiated plants in untreated soil) lithium was rapidly lost from the roots to the soil, suggesting that movement of lithium into and out of the plant depends—in part at least—on the direction of the lithium gradient.

The above work was carried out while the author held an appointment under the Hills Bequest (administered by the Royal Agricultural Society of England). The author wishes to express his appreciation of the interest shown by Prof. F. L. Engledow, and to acknowledge his gratitude to Prof. R. G. W. Norrish, of the Department of Physical Chemistry, who granted the use of a spectrograph, and to Dr S. Dickinson, who supervised the work and gave valuable advice.

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A REPORT OF PLANT REMAINS FROM MINNIS BAY, KENT

VII. DATA FOR THE STUDY OF POST-GLACIAL HISTORY

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(With 20 figures in the text)

THE material investigated was sent by Mr F.H. Worsfold from a Late Bronze Age hut site in Minnis Bay, near Birchington, North Kent. A small sample from each of three layers was examined. These were:

(*a*) A layer of peaty vegetable matter from the surface of hut site no. 11.

(*b*) Two samples of clay subsoil: an upper portion I, and a lower portion II.

The material was treated with 10 % nitric acid, sieved, and the products sorted.

A. Peaty vegetable matter

The material from the surface of the hut site yielded a good deal of plant material, consisting mainly of very worn, rounded pieces of wood about 5 mm. in length, several lengths of stems (up to 1 cm.), a short branch of a leafy liverwort, and a fragment of a leaf, besides several fruits and seeds.

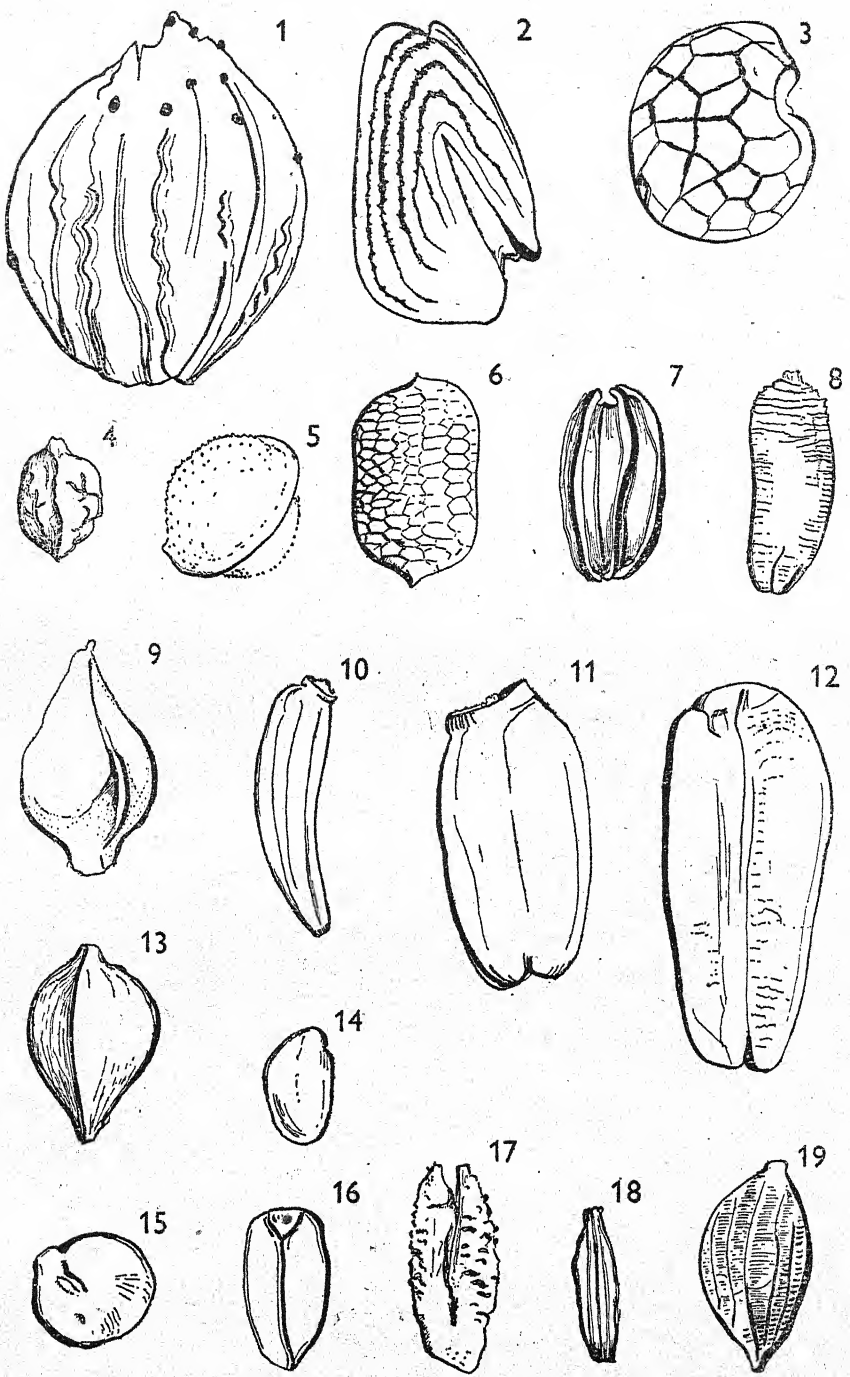
Among the seeds and fruits were good specimens of *Sanicula europea*, *Apium nodiflorum*, *Coriandrum sativum*, and *Picris echioides* in excellent state of preservation; several specimens of *Rubus*, *Potentilla*, *Alnus glutinosa*, *Potamogeton*, and *Carex* were recovered in bad condition.

Among the animal remains were numerous fragments of Bryozoa and Hydroids, and what appeared to be Tunicates; there were also several bits of chitinous shell, beetle elytra and egg cases. It is not clear how far these animal remains are related to the present situation of the peaty layer on the foreshore.

B. Upper part. I. Clay subsoil

This deposit yielded a fair number of seeds and fruits, including numerous specimens of various genera of Caryophyllaceae, *Carices*, and of *Rumex* and *Chenopodium*. Specimens were also obtained of *Papaver Rhoeas*, *Thlaspi arvense*, *Hypericum humifusum*, *Linum catharticum*, *Potentilla* spp., *Crepis virens*, *Calluna vulgaris*, *Lamium* sp., *Urtica dioica*, and *Juncus* spp. A few branches of mosses and several small fragments of other plant tissues were also recovered.

The animal remains included two specimens of ephippia of a daphnid, chitinous shell fragments, egg cases, beetle elytra, and several empty mite 'skeletons'.



Figs. 1–19. Seeds and fruits from Minnis Bay deposits. All enlarged × 30, except where otherwise stated. 1, *Coriandrum sativum.* 2, *Thlaspi arvense*, × 50. 3, *Papaver Rhoeas.* 4, *Rumex Acetosella.* 5, *Spergula sativa.* 6, *Hypericum humifusum*, × 100. 7, *Apium nodiflorum.* 8, *Picris echioides.* 9, Polygonaceae. 10, *Cnicus*, cf. *arvensis* (Lower II). 11, *Cnicus* sp. 12, *Cnicus*, cf. *lanceolatus* or *Cirsium anglicum* (Peaty Layer). 13, *Carex* sp. 14, *Linum*, cf. *catharticum.* 15, *Chenopodium* sp. 16, *Lamium* sp. 17, *Anthriscus vulgaris.* 18, *Crepis virens.* 19, *Juncus* sp., × 100.

Lower part. II. Clay subsoil

This portion contained far more seeds and fruits than the sample above, but many were in a bad state of preservation. There were again numerous specimens of Caryophyllaceae (especially *Arenaria serpyllifolia*), *Rumex Acetosella* et sp., *Chenopodium* sp., and *Carices*). The rest of the material included specimens of *Papaver Rhoeas*, *Thlaspi arvense*, *Hypericum humifusum*, *Linum catharticum*, *Potentilla* sp., *Cnicus* spp., *Crepis virens*, *Calluna vulgaris*, *Lamium* sp., *Urtica dioica*, and *Juncus* spp. A complete flower of *Trifolium procumbens* was also obtained (see Fig. 20), and the calyx and capsule of *Lythrum hyssopifolia*, and of a *Campanula* resembling *C. rotundifolia*.

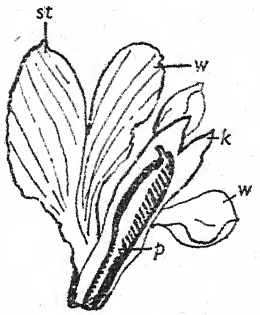


Fig. 20. Complete flower of *Trifolium procumbens* L. from the lower clay subsoil at Minnis Bay, *st*, standard; *w*, wing; *k*, keel; *p*, pod.

There were numerous animal remains including about eight specimens of the daphnid ephippia, besides material of the same nature as that in the clay sample next above.

Table 1 presents the identifications in a summarized form. Where not otherwise stated, the numbers apply to identifications of fruits or seeds.

General considerations

It is instructive to consider the list of determinations in the light of the conditions which these plant species now favour. Roughly speaking four habitats are represented: (1) cultivated and waste ground, (2) fresh water and fen, (3) sandy heath, (4) woodland. Species from the first habitat greatly preponderate. They include *Papaver Rhoeas*, *Thlaspi arvense*, *Arenaria serpyllifolia*, *Spergula sativa*, *Linum catharticum*, *Potentilla anserina*, *Anthriscus vulgaris*, *Coriandum sativum*, *Picris echioides*, *Crepis virens*, and *Urtica dioica*, together with a strong probability that the specimens from the genera *Ranunculus*, *Cerastium*, *Stellaria*, *Sagina*, *Cnicus*, *Lamium*, *Chenopodium*, *Polygonum* and *Rumex* belong very largely to this habitat also. It seems likely that this collection of species reflects the cultivation of crops by the Late Bronze Age inhabitants of the site, these plants possibly representing the residue from threshing. It should be noted that *Coriandrum sativum* and *Anthriscus vulgaris* have both been grown or collected for use in this country, and K. Jessen (1933) has recently made finds of seeds of *Spergula sativa* at Ginderup in Denmark, which show that the plant was used by Early Iron Age man, possibly as a food plant: it has been so used in Europe as late as the seventeenth century. The recognition of *Coriandrum sativum* is of particular interest to botanists, since this species is always considered to be alien to the British Isles. Hegi (1929), in reporting that it is a well-known and esteemed flavouring as well as a medicinal herb, says it has been known from ancient Egyptian times onwards, and indicates that its probable natural home in the eastern Mediterranean cannot now be established, since the plant is everywhere an inhabitant of cultivated ground. Its occurrence in Kent does not of course necessarily show the plant to have been *growing* locally,

Table 1

	Peaty vegetable matter	Clay subsoil	
		Upper layer I	Lower II
Ranunculus sp.	—	—	Several
Papaver Rhoeas L.	—	Eleven +	Ten
Thlaspi arvense L.	—	Three	One
Cerastium sp.	—	Several	Several
Stellaria sp. (cf. *media* Cyrill.)	—	Several	Several
Arenaria serpyllifolia L.	—	Very numerous	Very numerous
Sagina sp.	—	Few	Few
Spergula sativa Boenn.	—	—	One
Hypericum humifusum L.	—	Two	Three
Hypericum sp. (cf. *perforatum* L.)	—	—	One
Linum catharticum L.	—	One	Three
Trifolium procumbens L.	—	—	One complete flower
Rubus spp. (cf. *fruticosus* L. and *Idaeus* L.)	Several	—	—
Potentilla sp. (cf. *anserina* L.)	Few–several	—	—
Potentilla sp. (cf. *palustris* Scop.)	One	—	—
Potentilla spp.	Few?	Few, more than one sp.	One
Lythrum hyssopifolia L.	—	—	One perianth-tube
Sanicula europea L.	One	—	—
Apium nodiflorum Reichb.	One	—	—
Cf. *Anthriscus vulgaris* Bernh. (=*Chaerophyllum Anthriscus* Crantz.)	—	One	—
Coriandrum sativum L.	One	—	—
Cnicus spp.	One	—	Two
Picris echioides L.	One	—	—
Crepis virens L.	—	Three	Four
Campanula sp. (cf. *rotundifolia* L.)	—	—	One capsule
Calluna vulgaris Salisb.	—	Few	*c.* Four
Lamium sp.	—	Two	Two?
Chenopodium sp.	One	Very numerous	Numerous
Polygonum sp.	One	Several (cf. *Rumex*)	Several (cf. *Rumex*)
Rumex Acetosella L.	—	Numerous	Several
Rumex sp.	—	One fruit, several seeds	Several
Urtica dioica L.	—	Several	Several
Alnus glutinosa Gaertn.	Four	—	—
Juncus spp.	—	Six	Seven
Potamogeton spp.	Several	—	—
Scirpus sp.	One	—	—
Carex spp.	One	Numerous	Numerous
BRYOPHYTA			
Eurhynchium sp. (cf. *confertum*)	—	—	One leaf
Bryum sp.	—	—	Few leaves
Other mosses	—	Branches and leaves	—
Leafy liverwort	Branch	—	—
ANIMAL REMAINS			
Daphnid ephippia	—	Two	Several
Beetle elytra	+	+	+
Chitinous shell fragments	—	+	
Bryozoa	Numerous	One	—
Tunicata	+	—	—
Arthropod legs	—	+	+
Mites	—	+	+

but it establishes the strong likelihood that it could have become naturalized here as long ago at least as the Late Bronze Age.

A somewhat similar interest surrounds the whole list of species, for the origin of our common cornfield weeds and plants of waste places, and their place in a native undisturbed vegetation are perplexing problems to the phytogeographer. It will be noted that Matthews (1937) regards *Picris echioides* as having been introduced into Britain, and many others in the list have the same status. Thus Dunn (1905) puts the three common species of *Lamium* in this category, as well as *Thlaspi arvense*. The identifications given above afford at least positive evidence of the presence of many of these species already in south Britain in prehistoric time.

Fresh-water or fen conditions are indicated by the species *Apium nodiflorum*, *Alnus glutinosa*, *Potentilla* (cf. *palustris*) and the genera of *Juncus*, *Potamogeton*, *Scirpus* and *Carex*. All of these except *Juncus* are restricted to the upper peaty layer, which no doubt formed in fresh-water fen where these plants grew.

It is on the other hand only possible to regard as transported or derived the components of the two last groups, the woodland species, which include *Sanicula europea* and *Hypericum perforatum*, and the sandy heath species which include *Hypericum humifusum*, *Rumex Acetosella*, *Calluna vulgaris*, and *Campanula* (cf. *rotundifolia*). All of these except the *Sanicula* are found in the clay layers only.

The identification of *Lythrum hyssopifolia* is of interest chiefly on account of the present rarity of the species, which is still extremely rare in Kent as elsewhere.

The author is much indebted for direction and assistance to Dr H. Godwin.

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CHRYSOCHAETE, A NEW GENUS OF THE CHRYSOPHYCEAE, ALLIED TO *NAEGELIELLA*

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(With Plate 3 and 9 figures in the text)

CHRYSOPHYCEAE are still very incompletely known and nearly every habitat, within the range of their occurrence, can be shown on close examination to harbour some supposedly rare algae.

In 1892 Correns described a striking alga with brown chromatophores, *Naegeliella flagellifera*, found only once at Tübingen, Württemberg. This alga has not been recorded again since its discovery. It is a small epiphyte with a discoid colony of up to 150 cells, embedded in mucilage and bearing one or more branched or unbranched mucilaginous setae with multiple sheaths. A swarmer, observed in the same preparation, with two laterally inserted flagella, was believed to belong to this alga and is included in the diagnosis.

In 1927 Scherffel found a plant at Igló, Hungary, which, though similar in some general features, shows very considerable differences in the structure of vegetative cells, setae and swarmers. Scherffel described the plant as *Naegeliella* (?) and contemplated the need for a new genus. As only one certain swarmer was observed he decided that the time had not yet come to establish a new genus, though the swarmer was essentially different from that described by Correns. Scherffel established his form as a new species, *N.* (?) *natans*.

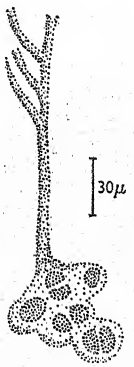
Another alga, similar to Scherffel's, was recorded by Godward in 1933. This shows significant differences from the plant described by Correns and less important differences from that of Scherffel. A new species, *N. britannica*, was established for it although no swarmers were observed. Godward found this alga in several widely separated localities, one might almost say wherever she looked for it. It must be considered to be a common epiphyte in the British Isles during the cooler months of the year.

Further information that has now accumulated makes it possible to clarify the rather complex relations between the algae described as three different species of the same genus. Good material was found in a greenhouse of the Biological Station at Lunz am See in Austria, as well as in various localities in the English Lake district during the winter. Many swarmers were observed and, as a result of careful comparison, it is thought necessary to establish a separate genus for Scherffel's and Godward's plants. It is proposed to call it *Chrysochaete*.

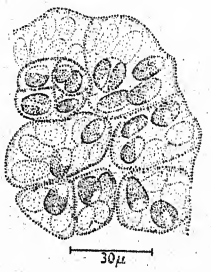
The main structural features of the algae will be discussed and diagnoses and

a key will be given in conclusion. Only brief reference need be made to the main points of interest, since the papers quoted give fairly comprehensive descriptions.

The habitats are essentially similar, the forms in question occurring in the shallow shore region of ponds or lakes. The community in which Correns found his plant consisted of *Cladophora*, *Apiocystis Brauniana*, *Mischococcus* Naeg., *Phaeothamnion*, etc. All these, with the exception of *Cladophora*, are mentioned also by the later authors, but in addition a considerable number of Chlorophyceae and Diatomaceae are enumerated as accompanying epiphytes. Submerged plants of all sizes may as a rule be colonized by Naegeliellaceae, but abundant growth has also been observed on glass slides suspended in the water (Godward, 1933). Scherffel found his form frequently floating on the water surface, as well as epiphytic on *Vaucheria*, and regards the floating habit as characteristic for the species. It seems, however, that this is not significant, as the plant studied by the writer, which is identical with that described by Godward, was frequently found on the water surface. It was easily obtained on cover-slips supported by corks on the surface



Text-fig. 1. *Chrysochaete britannica*. Young colony grown on agar medium; cells lie far apart in mucilage.



Text-fig. 2. *Chrysochaete britannica*. Part of old colony (see Pl. 3, fig. 2), showing mucilage investments.

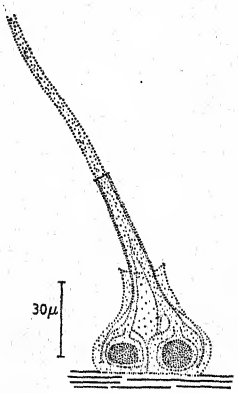
film of the tank containing the material. It seems clear that several factors combined are responsible for the direction in which the swarmer moves and where, consequently, the new colony develops.

The alga appears to tolerate a wide range of waters from such as are very rich in nutrients like the greenhouse tank with abundant growth of mainly *Salvinia natans* on the submerged leaves of which the alga was found, to those very poor in such substances, as of some of the English lakes. The statements of certain authors that Naegeliellaceae are abundant or solely represented during the cooler months, can be confirmed for Windermere. The Lunz record, however, contradicts this, as the alga was found in a hothouse in August. This shows that temperature as such cannot be of decisive importance, but that under natural conditions the combination of factors typical for the colder months is favourable for the development of Naegeliellaceae.

The shape of the colonies of the Naegeliellaceae varies considerably in corre-

spondence with the surface on which it occurs. The usual shape is that of a more or less irregular disk (Pl. 3, figs. 1, 2), but according to Correns the colony of *Naegeliella* may encircle a *Cladophora* cell and thus assume a cylindrical shape. Large colonies are the exception; Correns refers to a maximum of about 150 cells, Godward to 50, while in Lunz up to about 320 cells forming a compound colony, 204μ in diameter, were observed (Pl. 3, fig. 2). The cells are embedded in mucilage and may be closely packed as a result of frequent divisions when they are flattened against each other; in other instances they may lie far apart in the mucilaginous matrix. This has been observed in nature and in cultures on agar (Text-fig. 1). In old colonies mucilage investments are clearly recognizable round individual cells and their products of division (Text-fig. 2). This is, however, a variable feature depending on the division rate and cannot be used as a specific distinction. Godward's reference to this on p. 41 is therefore not valid. Scherffel observed conical, stratified mucilage covers in several cases and refers to mucilage envelopes on p. 384. All observers, except Scherffel, record division in three planes in large colonies (Text-fig. 2). This author gives the maximum cell number observed in a colony as 6, which explains the difference.

The striking mucilage setae, which are so characteristic of the family, are often not easily visible. Considerable confusion of terms exists with regard to this structure which is a non-septate, non-cellular elongation of the mucilage covering the colony. The term 'hair' should be reserved for structures such as are common among Chaetophorales (e.g. *Chaetophora*), which consist of one or more cells. All the other manifold structures should be termed 'setae', when it is a question of differentiation of a cell or part of it only. Such use of the terms would reduce existing confusion considerably. A similar delimitation has been made by Möbius (1892) and Huber (1892). The structure of such setae may be very complicated and may vary considerably in different genera or even species of the same genus.

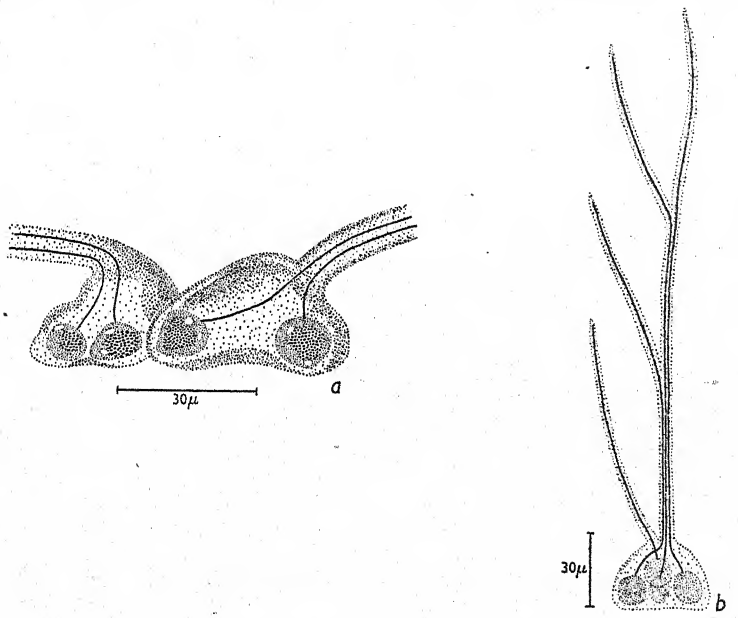


Text-fig. 3. *Naegeliella flagellifera*. Young colony, showing structure of seta. Redrawn after Correns.

Correns described the structure of the seta in *Naegeliella* in great detail (Text-fig. 3). It consists of a number of consecutive mucilaginous sheaths, one within the other, the outermost being the mucilage layer covering the cell. In a very young colony the mucilage covering the cell is pierced by a papilla which in its turn is penetrated by the seta proper which grows through this double sheath. As the cell divides the seta of the unicellular colony is pierced by two further setae, while it itself now functions as a sheath enveloping the two newly formed setae. This process is repeated as the colony increases in number of cells and results in the formation of thick bundles of setae, which may split into several units, such as protrude from an old colony. Such increase of the outer sheaths, as well as the growth in length of the setae, can only be understood, according to Correns, as due to growth by intussusception which probably takes place throughout their

whole length. Correns found no evidence of a lumen or of cytoplasm inside the seta. So far as is known, growth by intussusception occurs only when there is increase of an existing structure and not when a new one is formed in which case the presence of protoplasm is regarded as essential. The matter requires further study in *Naegeliella.*

The absence of a protoplasmic thread in the setae of *Naegeliella* must be emphasized, as this is one of the essential differences from the other plants under discussion. It is important to remember that Correns was the discoverer of protoplasmic threads in the setae of *Apiocystis* and that he considered the possibility of the presence of such a thread in *Naegeliella.* This, together with the very high standard of accuracy of his work, justifies the view that Correns is unlikely to have



Text-fig. 4. *Chrysochaete natans.* (*a*) Young colonies, protoplasmic threads; one originating from each cell extending into seta. (*b*) Structure of branching seta in a four celled colony; diagram, redrawn after Scherffel.

overlooked the protoplasmic thread in *Naegeliella.* The other authors, who have studied members of Naegeliellaceae, agree as to the presence of a protoplasmic thread extending from the cell through the seta and, in favourable cases, traceable to its very end.

Scherffel's figures in my Text-fig. 4 clearly indicate the presence of such threads, presumably one for each cell of the colony. Up to ten setae were observed on a single colony. They break off fairly easily so that many cells appear to lack them. On the other hand, exceptional instances have been found, where one cell may produce two or even three protoplasmic threads. This is probably due to inhibition of division of the protoplast. Scherffel is of the opinion that the thread enters the seta only after the mucilage sheath has started to grow. It would seem, however, much more

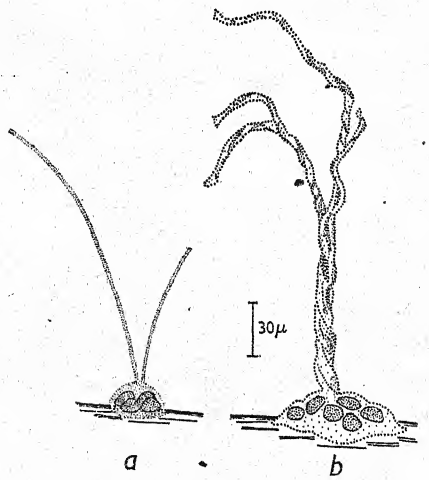
probable that the mucilage is produced secondarily and after the formation of the protoplasmic thread. It has been impossible to follow this in the Naegeliellaceae, but evidence from other families may be quoted to show that, where carefully studied, development is initiated by the protoplasmic thread and not vice versa.

In *Apiocystis Brauniana* the protoplasmic thread originating from the posterior end of the cell may sometimes even grow to its full length before the mucilaginous sheath is developed (Correns, 1893). The mucilage is apparently produced only on contact with water, although the presence of the protoplasmic thread is essential. The result of this process is a seta somewhat similar to that observed in the Naegeliellaceae, excluding Correns's plant.

Cytological evidence for the primary importance of the protoplasmic thread from the morphogenetic point of view is given by Wesley (1928) for *Coleochaete scutata*. It is shown that the protoplasmic thread during the initial stages of seta formation originates from a granule (her 'blepharoplast'), closely connected with the nucleus. The further development of the seta consists in the production of a mucilaginous sheath which is pushed forwards by the protoplasmic thread; at a later stage the latter pierces the sheath and grows out uncovered into the water.

Diverse members of Tetrasporales, Chlorococcales, and Chaetophorales show somewhat similar setae to those of the Naegeliellaceae. In all cases studied in greater detail, the setae remain intact after swarmers have escaped and new ones develop, where this has been determined, from the posterior end of the cell (Correns, 1893; Geitler, 1923; Hieronymus, 1890; Pascher, 1929).

Though frequently visible in vivo (Scherffel and the writer) the plasmatic thread shows up better after staining. The methods used by different authors to study the structure of the setae in the Naegeliellaceae usually consist in staining with methylene blue; iodine green followed by dilute potash, and other stains were also employed.



Text-fig. 5. *Chrysochaete britannica*. (*a*) Unequal development of setae on young colony. (*b*) Compound seta; twist appears after staining, indicating a possible spiral structure of mucilage.

The length of the setae ranges from 150 to 400μ, the longest so far observed. The material from Lunz and Windermere agrees in all essential points with that of Godward. Pl. 3, fig. 3, is a photograph of a medium-sized colony in an indian ink suspension, showing up the compound seta.[1] The number of setae on a single colony seems to vary considerably and is not subject to any general rule. The small colony in Text-fig. 5*a* already has two, that in Text-fig. 5*b* at least four setae. Whether

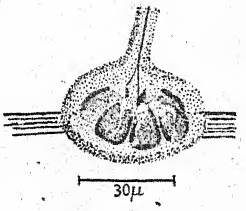
[1] The indian ink used is 'Pelikan' indian ink for biological studies, after A. Niklitschek, supplied by L. and C. Hardtmuth, Stafford Rd., Croydon. Living cells are hardly damaged at all by this preparation.

the primary setae remain united in a single bundle or whether they stand out separately depends largely on the rigidity of the primary sheath. Fifty-six colonies were examined in order to determine the distribution of setae. The following table shows that 26 % of the colonies had no setae at all, which was probably due to their having broken off:

No. of cells per colony	No. of setae		
	0	1	2
2	6	5	0
3	1	3	0
4	6	24	4
5	1	1	0
6	1	1	0
7	0	1	0
8	0	2	0

The figures indicate the number of colonies showing 0, 1 or 2 setae respectively.

The protoplasmic thread could often be seen clearly in the living alga. It was not possible to detect all the threads in a seta because of overlapping of cells and threads. In several cases the origin of the protoplasmic thread was clearly seen. After cell division the appearance shown in Text-fig. 6 was frequently observed, each daughter cell having formed a new basal section to the protoplasmic thread of the mother cell, so that the lower end of the thread appears split. It is not known whether this split later extends upwards and results in two separate threads reaching to the end of the seta or whether, when cell divisions are very frequent, the number of threads in a seta may be smaller than the number of cells, in an extreme case only one with short basal connexions to each cell. Godward mentions such an instance in her form and regards it as a definite difference from Scherffel's plant. The latter, however, figures separate protoplasmic threads only in a colony of two cells, and it is not clear whether he actually observed separate threads in larger colonies. His Fig. 53 is a diagram (see Text-fig. 4*b*). All observers agree that the setae frequently show branching in the upper part; in most cases this is dichotomous. The protoplasmic thread itself, however, shows no branching. From all that is known the presence or absence of a protoplasmic thread must be regarded as of great importance since its formation is the result of a complicated, fundamental process, which is of special value from a systematic point of view in a group in which distinctive characters are scarce.



Text-fig. 6. *Chrysochaete britannica*. Basal section of protoplasmic thread in young colony, newly formed by daughter cells after division.

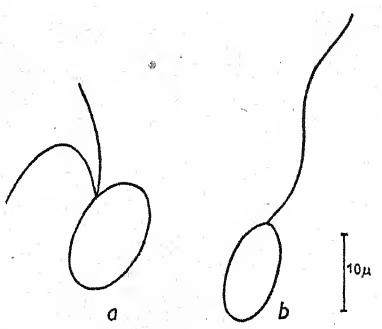
The structure of individual cells in the different forms is remarkably similar and it would be almost impossible to distinguish between isolated vegetative cells. The range of size is given by Correns as 9–14 × 11–16μ, by Scherffel as 6–10 × 12–14μ, and by Godward as 4–9 × 10–15μ; in my form it is 4–8 × 8–17μ. In shape the cells vary from spherical to long and narrow. There is one golden brown, plate-shaped and sometimes lobed chromatophore which is frequently bent into a

V shape; it may be somewhat removed from the cell periphery, although normally parietal in position. Just before cell division two distinct chromatophores can be seen. There is no pyrenoid in any of the forms under consideration. No starch could be detected in the cells, but oil droplets and leucosin are present (Text-figs. 8, 9), and have been recorded by all authors. The protoplasm is usually remarkably clear and transparent and shows practically no granules. The nucleus could not be seen in the living cell.

Of great interest are the contractile vacuoles observed by all except Correns. They are always present but not strictly localized, and their number varies from 1 to 5. Scherffel recorded 4 or 5, Godward 2 to 4, while the writer observed 1 to 2 only. The difference in number is probably due to different physiological conditions and is not a constant specific difference, since variation could be observed from cell to cell. The vacuoles are simple, contracting periodically, with complete expulsion of their contents.

Cell division may take place in three planes, and results in old colonies of a thickness of several layers.

The motile stage, which is of great importance for the identification and description of microscopic algae, is mainly responsible for the establishment of a second genus of the Naegeliellaceae. The Chrysophyceae as a whole, although structurally a unit, show a great variety of motile types. If classification in this class were based on similarity of swarmers it would result in an absurd and unnatural classification. The motile stages have therefore to be considered in conjunction with other characters, and this principle has been applied to a number of families within the class, with the result that genera with swarmers of different structure are placed in one family. Species with different types of swarmers cannot, however, be included in a single genus.



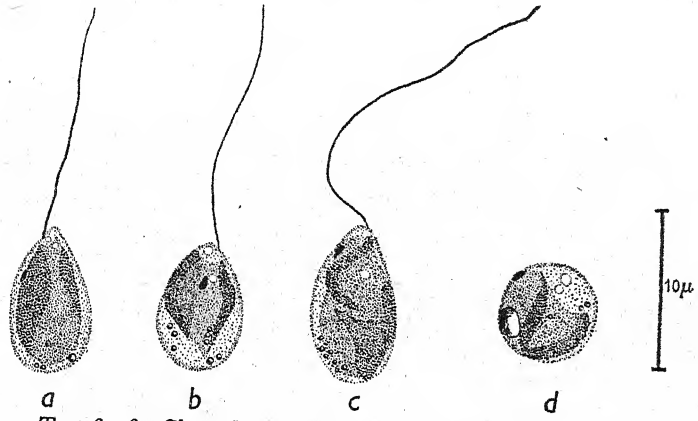
Text-fig. 7. (*a*) *Naegeliella flagellifera*, swarmer. (*b*) *Chrysochaete natans*, swarmer. Redrawn after Correns and Scherffel.

Correns mentions that all vegetative cells may become motile, and he observed one swarmer actually emerging through the mucilage, but this was unfortunately lost before its structure could be studied. Other swarmers present in the preparation were described as those of *Naegeliella*. These must, however, be treated with a certain reserve, as contamination with another species cannot be entirely excluded. These swarmers were ovoid, 15μ long and possessed one chromatophore but no stigma; oil droplets were present. The most important character are the two laterally inserted flagella (Text-fig. 7*a*) which are typical for *Ochromonas*-like swarmers. Correns alone records divisions in some cells of the colony which he suggests might be early stages in microzoospore formation. No motile cells were, however, observed.

The type of swarmer described by Correns provides the difficulty in allotting

Naegeliella the right systematic position. Pascher in 1913 and Oltmanns in 1922 placed it in the Phaeocapsales among the Cryptomonadales, where it remained until 1925, when Pascher transferred it to a monogeneric family among the Chrysocapsales. This position was adopted by Fritsch (1935). Later observations on the motile stages of the other forms made their position among the Chrysocapsales quite clear.

Scherffel observed one swarmer only, emerging from the colony. It appears—although this is not quite clear from the description—that this was not the result of a division, but rather a whole protoplast transformed into a swarmer. Observation was possible only for a short time, and this probably accounts for the very incomplete outline drawing (Text-fig. 7*b*) by an author who is well known for the excellence of his illustrations.[1] Amoeboid movement is recorded, while the swarmer penetrates the mucilage. A single chromatophore without a stigma, and one long apical flagellum were recorded. This is in striking contrast to the swarmer described



Text-fig. 8. *Chrysochaete britannica*. (*a–c*) Swarmers, (*d*) swarmer settled down, containing oil and leucosin.

by Correns. Godward found a number of empty cells, which were also observed by Scherffel, their envelopes torn at the top, which were taken to indicate the escape of swarmers. No motile stages were, however, observed. Scherffel's findings agree in principle with the writer's observations.

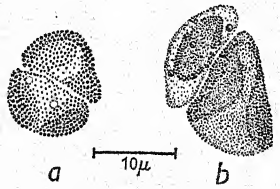
No divisions precede swarmer formation at any time. The first indication of the imminence of the motile stage is the appearance of a bright orange-red stigma at a time when the cell shows no other changes. The contractile vacuoles remain in evidence, usually numbering two. The protoplast moves slightly while still in its original position and then suddenly squeezes through the overlying mucilage which has now become diffluent. A short period of amoeboid movement follows, but the final shape is soon assumed. The swarmer is ovoid and slightly flattened with an obliquely truncate anterior end. One large golden brown parietal chromatophore is present, with inturned edges (Text-fig. 8 *a*, *b*) or showing more complicated

[1] No measurements are given, but by comparison with other figures a length of 13 μ is likely to be correct.

foldings (Text-fig. 8*c*). In most cases it lies so close to the periplast that no cytoplasmic layer is distinguishable beyond it. Oil droplets are always present and usually accumulate in the posterior end of the cell. Occasionally large balls of leucosin can be observed, also situated in the posterior end (Text-fig. 8*d*). The quantities of these two substances vary with the physiological state of the cells and must not be regarded as a constant characteristic. A very distinct orange-red eyespot is always situated near the anterior end, in close contact with the chromatophore (Fig. 8). It remains visible for a short time after the swarmer has settled down, but does not survive the first cell division. There are always two contractile vacuoles mostly situated very near the anterior end but sometimes nearer the middle of the cell, overlying the chromatophore. After the first division they lie anywhere in the cell.

After the period of amoeboid movement, which may, however, reoccur at a later stage, the flagellum can be studied. While the length of the cell ranges from 10 to 14μ the flagellum may attain a length of up to 25μ. It is inserted a little towards one side at the base of the short truncate anterior end of the cell. The flagellum exhibits slow undulations which result in a forward movement with rotation round the longitudinal axis. The motile stage seems to last for a short time only, and this is probably also so under natural conditions, as free swarmers are rarely encountered, except at times when they are actually seen emerging. The swarmer is of the Chromulina type.

When the swarmer settles down, it assumes a spherical shape and the flagellum disappears. The result of the first division is seen in Text-fig. 9, and subsequent divisions lead to the formation of a colony as described above. As soon as a two-celled colony is formed, a seta may be produced.



Text-fig. 9. *Chrysochaete britannica*. Two celled colonies, result of first division; stigma has disappeared.

As regards the systematic position of the four forms under discussion there is no doubt that they must be included in the Chrysocapsales, the order corresponding to the Tetrasporales in the Chlorophyceae. The structure of the setae in the Naegeliellaceae is an additional detail confirming parallel development in the two classes, although it is more complicated than that in the Tetrasporales.

From the foregoing description it is clear that two genera must be distinguished. One is *Naegeliella*, with the single species, *N. flagellifera* Correns, the other *Chrysochaete*, n.gen. including two species, *C. natans* (Scherffel) Rosenberg and *C. britannica* (Godward) Rosenberg.

The following key should help in identifying the different forms:

Microscopic, epiphytic algae, colonies embedded in mucilage, one or rarely more layers of cells. No cell membrane. One plate-shaped chromatophore, oil droplets and leucosin. Long mucilage setae, one to ten on each colony, consisting of a number of consecutive sheaths, setae dichotomously or irregularly branched. Increase of colony by repeated cell divisions. Reproduction by swarmers. Cell dimensions 4–16 × 8–17μ.

NAEGELIELLACEAE, 2 genera

1. Setae with central protoplasmic thread, usually one to each cell, surrounded by mucilage sheaths. Motile cells ovoid, obtusely truncate apex, 1 flagellum twice as long as cell or longer, laterally inserted. Contractile vacuoles 1–5 in vegetative cells and swarmers. **Chrysochaete**, 2 species.

2. Setae without central protoplasmic thread, consisting of a series of consecutive mucilage sheaths, pierced by the setae. Motile cells ovoid, non-truncate apex, 2 laterally inserted flagella of unequal length, no stigma. No contractile vacuoles. Cells 11–16μ long, 9–14μ wide, zoospore 15μ long.
Naegeliella, 1 species, **N. flagellifera** Co.

Chrysochaete

1. Colonies 2–6 cells, covering mucilage stratified. 4–5 contractile vacuoles. Zoospore non-truncate apex, without stigma. Cells 6–10 × 12–14μ. Zoospore about 13μ long.
C. natans (Scherffel) Rosenberg.

2. Colonies 2–320 cells, covering mucilage not stratified, old colonies 2 or 3 cell layers thick. 1–4 contractile vacuoles. Zoospore with obtusely truncate apex, orange-red stigma. Cells 4–9 × 8–17μ, zoospores 10–14μ long, flagellum usually 2 cell lengths, maximum length 25μ. **C. britannica** (Godward) Rosenberg.

Latin diagnoses of the three species follow:[1]

Naegeliella, nov.gen. Correns

(translation of Correns's diagnosis)

Cellulae ovoideae, nucleo uno, chromatophoris magnis flavo-fuscis (Diatominum continentibus); sine pyrenoide vel amylo, guttis oleae praeditae; mucum producentes. Per divisiones cellulae familiam discoideam, primo e strato singulo cellularum, deinde e pluribus stratis constantem, rotundam vel ovatam, epiphyticam formantes. Familiae setis mucosis longis, singulis vel pluribus, simplicis vel ramosis praeditae. Reproductio zoosporis monosymmetricis, quae cellulae somaticae familiae, per imbibitionem muci liberatae sunt; flagellis duobus, lateraliter insertis, sine stigmate, quietis novam familiam formantibus.

Dimensiones: cellulae 11–16μ longae, 9–14μ latae; zoospora 15μ longa.

Habitat on *Cladophora* in a small pond in the Botanic Garden, Tübingen, Württemberg, Germany. Found in the autumn. Only record.

Chrysochaete, nov.gen. Rosenberg

Familiae epiphyticae, discoideae, in plantis aquaticis vel laminis vitri. Familia e cellulis 2 ad 310 constans, strato gelatinoso tecta; cellulae singulae vel nonnullae, integumentis gelatinosis praeditae; setae longae mucosae, in familiis juvenalibus e tubo singulo constantes, in familiis senioribus, elaboratae valde ramosae, saepe furcatae, e tubis mucosis plurimis constantes; filum protoplasmaticum in quoque tubo unum, e cytioplasmate cellularum quarumque ortis, in vivo conspicuum. Chromatophora unum vel duo, flavo-fusca, saepe curvata in forma V, cellulae guttis oleae et leucosine praeditae, sine pyrenoide; 1 ad 5 vacuolis contractibilibus.

[1] The help of Dr E. F. Warburg with the translation of these diagnoses is gratefully acknowledged.

Reproductio zoosporis uniflagellatis, similibus cellulis somaticis mobilibus, flagellum longitudine duarum cellularum vel longius. Divisiones ante formationem zoosporarum non observatae. Zoosporae liberatae per imbibitionem muci, primo modo amoebae deinde per flagellum moventes; quietae novas familias formantes.

2 species.

1. *C. natans* (Scherffel) Rosenberg (=*Naegeliella* (*?*) *natans* Scherffel).

Familia e 2–6 cellulis constans, strato gelatinoso laminato; setae raro 2 vel 3 filis protoplasmaticis ex una cellula ortis praeditae; cellulae quaeque vacuolis contractibilibus 4 vel 5 praeditae; zoospora apice uniflagellata, sine stigmate.

Dimensiones: cellulae 6–10μ × 12–14μ, zoospora 13μ longa, setae 2–4μ latae.

Habitat on *Vaucheria* and free floating on water surface film, Igló, Hungary. Only record.

2. *C. britannica* (Godward) Rosenberg (=*Naegeliella britannica* Godward).

Familiae 2–320 cellulis constans; strato gelatinoso 3–4μ lato, non laminato tecta; cellulae stratum singulum sed in parte centrale familiarum vetustarum strata duo superposita formantes; setae 1–10 in familia; fila protoplasmatica nonnumquam basin versus furcata ad cellulas duas juncta; cellulae globosae vel elongatae, 1–4 vacuolis contractibilibus praeditae; stigmate ante formationem zoosporae patente; zoospora paulo complanata, apice obtuse truncata, stigmate aurantiaco-rubro, ad chromatophorum affixo; vacuolis contractibilibus duobis versus apicem praedita.

Dimensiones; familia 204μ diametro maximo; setae 150–400μ longae, 3μ latae; cellulae 4–9μ × 8–17μ; zoosporae 10–14μ longae, flagellum maximum 25μ.

Habitat in shallow region of lakes and ponds, epiphytic on aquatic plants, also observed on glass slides South England and English Lake district, on *Salvinia* in a greenhouse tank, Lunz, Austria. Distribution widely scattered, common in cooler months.

My thanks are due to Prof. F.E. Fritsch, F.R.S., for reading the manuscript and for helpful suggestions.

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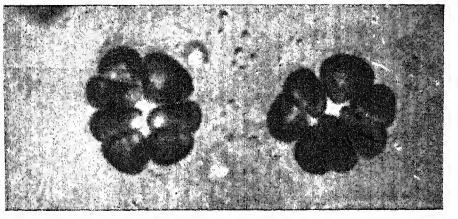

Fig. 1

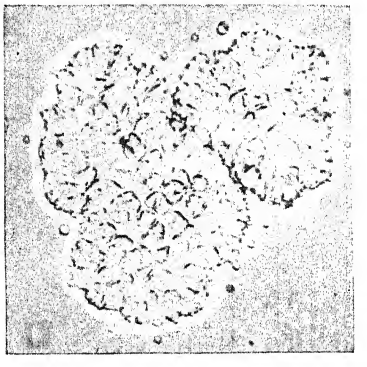

Fig. 2

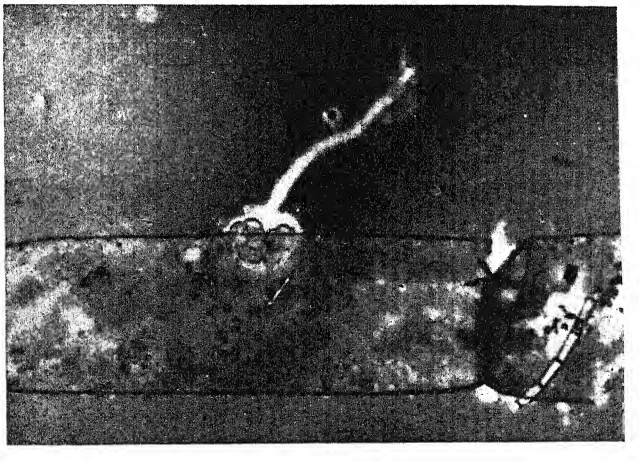

Fig. 3

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EXPLANATION OF PLATE 3

Chrysochaete britannica (Godward) Rosenberg

Fig. 1. Young colonies; magnification 500×.
Fig. 2. Old compound colony showing mucilage investments and mucilage cover. Living material in special indian ink suspension; magnification 225×.
Fig. 3. Young colony on *Salvinia natans*, showing mucilage cover and seta. Living material in special indian ink suspension; magnification 225×.

SOME OBSERVATIONS ON *CYSTOSEIRA FOENICULACEA* (L.) GREV. EMEND. SAUVAGEAU

By A. E. ELSE DAWSON
Westfield College, University of London

(With 2 figures in the text)

The genus *Cystoseira* is so well known, and its oospores are so favoured as material for experiments on polarity and other matters, that one or two new features brought to light may be of interest. It is one of the most familiar northern representatives of the Sargassaceae and usually a dense colonizer of calm tidal pools. The present species flourishes locally on both sides of the English Channel. For my material I am indebted to Mrs E.M. Laing, M.Sc., who collected at Perros-Guirec (Brittany) in August 1939 and fixed fertile fronds in formalin-acetic-alcohol and in formalin-sea water. The collector's description of the complete plants, given verbally at the time, tallies with the accounts of De Toni (1895) and of Newton (1931) for *C. discors* (L.) Ag. These writers used the name *C. discors* comprehensively, but Sauvageau (1911–12), with his wide knowledge of *Cystoseira*, distinguished three species: the Mediterranean *C. discors* (L.) Ag. emend. Sauvageau, *C. myriophylloides* Sauv., and the Atlantic *C. foeniculacea* (L.) Grev. emend. Sauv. Comparison with a number of herbarium specimens, including those of Sauvageau made available in difficult circumstances at the British Museum (Natural History) through the kindness of the Keeper of Botany, indicates the identity of my material with the last of these.

The bushy frond reaches a length of about 80 cm. Its flattened leafy basal parts grade into radially constructed branches in which intercalary air vesicles are scattered, often subtending the terminal, forked, fusiform receptacles (see Harvey, 1871, Plate CXXII).

There are many cryptostomata, and conceptacles of three kinds: most are bisexual, with the oogonia interior and the antheridia in a ring below the ostiole. But some conceptacles are entirely male and a few produce only oogonia. This accords with Sauvageau's observation that in a group of five species: *C. foeniculacea*, *C. discors*, *C. abrotanifolia*, *C. myriophylloides* and *C. canariensis* ('les *Cystoseiras* à anthérozoïdes sans point rouge', 1911*b*) there is a trend towards segregation of the sexes and reduction in the total number of antheridia. The various stages in the development of the conceptacle in the present material show that *C. foeniculacea* augments the growing list of Sargassaceous species which corroborate Nienburg's description (1912) for *C. barbata*. One receptacle holds two dozen or more conceptacles which are wart-like from the outside and are formed acropetally in close succession.

Development in the conceptacle

(*a*) *Hairs.* The floor of the conceptacle gives rise to long, basally growing filaments which straggle out of the ostiole (Fig. 2 G, *h*), and the sides produce short, branched hairs, like typical fucaceous antheridial tufts. A central sheaf of long sterile hair was seen by Sauvageau in the conceptacles of his five closely allied *Cystoseiras* (1911, 1912). He regarded such conceptacles as transitional from cryptostomata. Observations of short concrescent tufts in *Carpophyllum flexuosum* (Dawson, 1940), *C. maschalocarpum* (Delf, 1939*a*) and *Xiphophora chondrophylla* (Mitchell, 1941) probably indicate a corresponding growth. Very different in fate, but like in origin, is the central mass of tissue which grows out of a conceptacle, to continue as a branch, in the parasite *Notheia anomala* (Gruber, 1896; Williams, 1923). It is interesting that the only other record of this kind of branching is for a salt-marsh form of *Fucus ceranoides* (Skrine *et al.* 1932). These growths all testify to the trichothallic potentiality of fucoids, particularly when living in unusual conditions.

(*b*) *Antheridia and oogonia.* The antheridia are usually practically sessile (Fig. 1 C, a_1), and fresh ones proliferate through emptied sheaths. Sauvageau's record (1911*b*) of the absence of any eyespot indicates reduction in the free-swimming independence of the spermatozoids, as contrasted with those of the majority of species of *Cystoseira*. The oogonia like the antheridia ripen successively, the younger ones growing up beside or through the relics of their predecessors. This contrasts with the single and limited fertile period of the conceptacles in *Sargassum* (Tahara, 1909, 1913) and in *Carpophyllum* (Delf, 1939*a*; Dawson, 1940). As few as two or, more typically, as many as twelve oogonia develop in one conceptacle.

The differentiation of an individual oogonium follows the periclinal division of a superficial cell into an inner cell and a papilla (Fig. 1 B). One or more divisions of the latter may occur before the lowest cell of the series produced enlarges conspicuously (Fig. 1 C). Thus the oogonium itself is ontogenetically an intercalary cell, sometimes on a unicellular stalk and it bears distally a short, single or branched mucilaginous hair (Fig. 1 D). Comparable behaviour has very rarely been described. In *Notheia* Gruber tacitly figured oogonia supporting multicellular hairs (Fig. 9, Table II, 1896), and Oltmanns (1922) specifically noted that the former basal cells became oogonia. The oogonia of *Sarcophycus* (= *Durvillea*) *potatorum* also grow on filaments, but terminally (Whitting, 1893). Trichothallic growth prevails here and there in the Fucales, particularly where conservative organs such as those of reproduction are concerned; and these observations show that the oogonia like the antheridia may in all genera have developed originally from hairs. *Cystoseira*, notable for its morphological and geographical plasticity, seems to show at a primitive or experimental stage various trends which are consummated in other genera. As the oogonia enlarge, their hairs often become displaced or destroyed leaving quite typical, almost sessile, organs surrounded by independent paraphyses (Fig. 1 A).

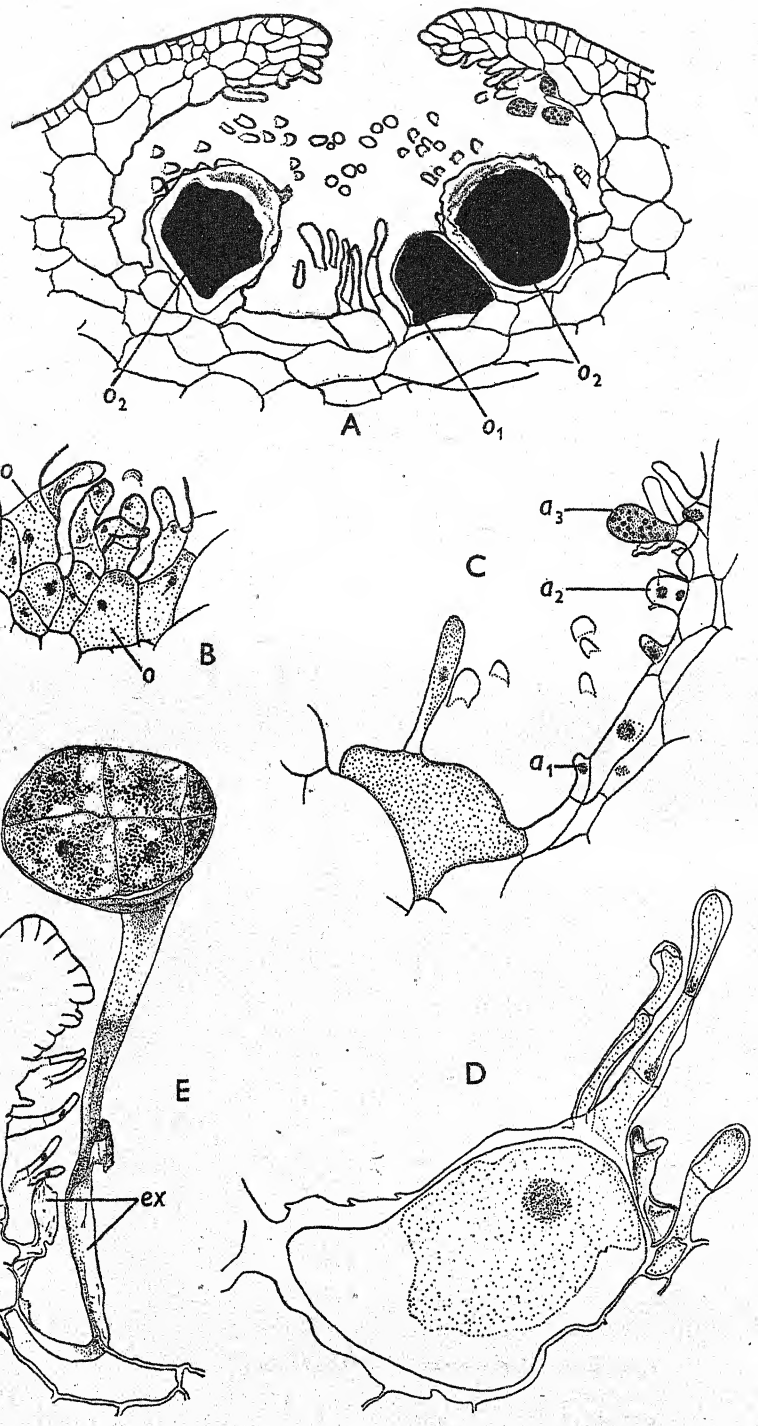

Fig. 1. *Cystoseira foeniculacea* (microtome sections). A. L.S. conceptacle (nearly median; ×150). B. Lining of conceptacle with very young oogonial filaments (*o* = young oogonium ×420). C. Lining of conceptacle with older oogonium and proliferating antheridia (a_2, a_3; ×420). D. Large piliferous oogonium (×420). E. L.S. sporeling surrounded by attenuated sheath and attached to inside of conceptacle (*ex* = exochiton; ×220).

Oogonial stalk formation

In preparation for the escape of their contents the oogonial walls produce specialized slime. The originally even, thin apex (Fig. 1A, o_1) thickens (Fig. 2A) as mucilage is secreted inwards towards one side, rendering the protoplast asymmetrical (Fig. 1A, o_2). Successive mesochitonous layers develop between the exochiton and the protoplast forming a closely confined column which curves to one side (Fig. 2B). Pressure apparently causes the end to adhere to the inside of the exochiton, and the junction is so firm that when a mature stalk is removed from the parent conceptacle it drags the exochiton away with it (Fig. 2F).

I have not followed the development of the column into a functioning stalk while alive, but have been able to initiate it by treatment of well-preserved material. The method was to transfer conceptacles gradually from alcohol to dilute glycerine and then to dissect out oogonia from conceptacles whose partial evacuation indicated ripeness. The largest oogonia were placed in sea water and the apex of the exochiton pierced with a needle. The mucilage now bulged out slightly (Fig. 2C), and when the sea water was diluted with distilled water the emerging mass swelled and revealed itself as a firm strand (Fig. 2D). The actual escape of the contents had usually to be assisted by peeling off the remaining exochiton (Fig. 2E), probably because the fixation of the material in this unripe state prevented the further swelling of the maturing oosphere which would be expected to accelerate natural emergence. Finally, the extending strand straightened itself out (Fig. 2F). Its three or four segments correspond to the original layers of mucilage laid down, whose proportions change from flattened to elongated cylinders. The core is slightly denser, and the whole structure is so tough that it withstands stretching to half again its normal length and considerable prodding. Within the conceptacle the tapering end of each stalk is firmly attached to the inside of its parent exochiton, as described above (Fig. 1E), while the thicker part of the sheath may be constricted as it traverses the ostiole. The exochiton persists in situ as a transparent fluted cup. There is an apparent discrepancy between Figs. 1E and 2F because the latter is from a dissection made soon after the arrival of the material, and shows all the differentiation described, while Fig. 1E, with a more homogeneous stalk, was drawn after embedding and cutting the receptacle.

Evidence of oogonial periodicity in *Cystoseira* is inconclusive. Sauvageau (1912) was undecided about this point but described zones of the conceptacles wrapped in sheathed oospheres, e.g. in *C. discors*. In the present material I have not seen the localized bands of shed oospheres characteristic of *Sargassum* and *Cystophyllum* (Tahara, 1913), and incline to the belief that periodicity is only incipient in *Cystoseira foeniculacea*. Thus the absence of massed mesochitonous sheaths in the majority of fertile herbarium specimens examined suggests that there may be restricted times of shedding. The closely parallel stages of embryogeny in my material may mean that many conceptacles dehisce simultaneously. The number of developmental stages within and without a single conceptacle provoke the suggestion that this synchronous extrusion is repeated once or twice. In this sense

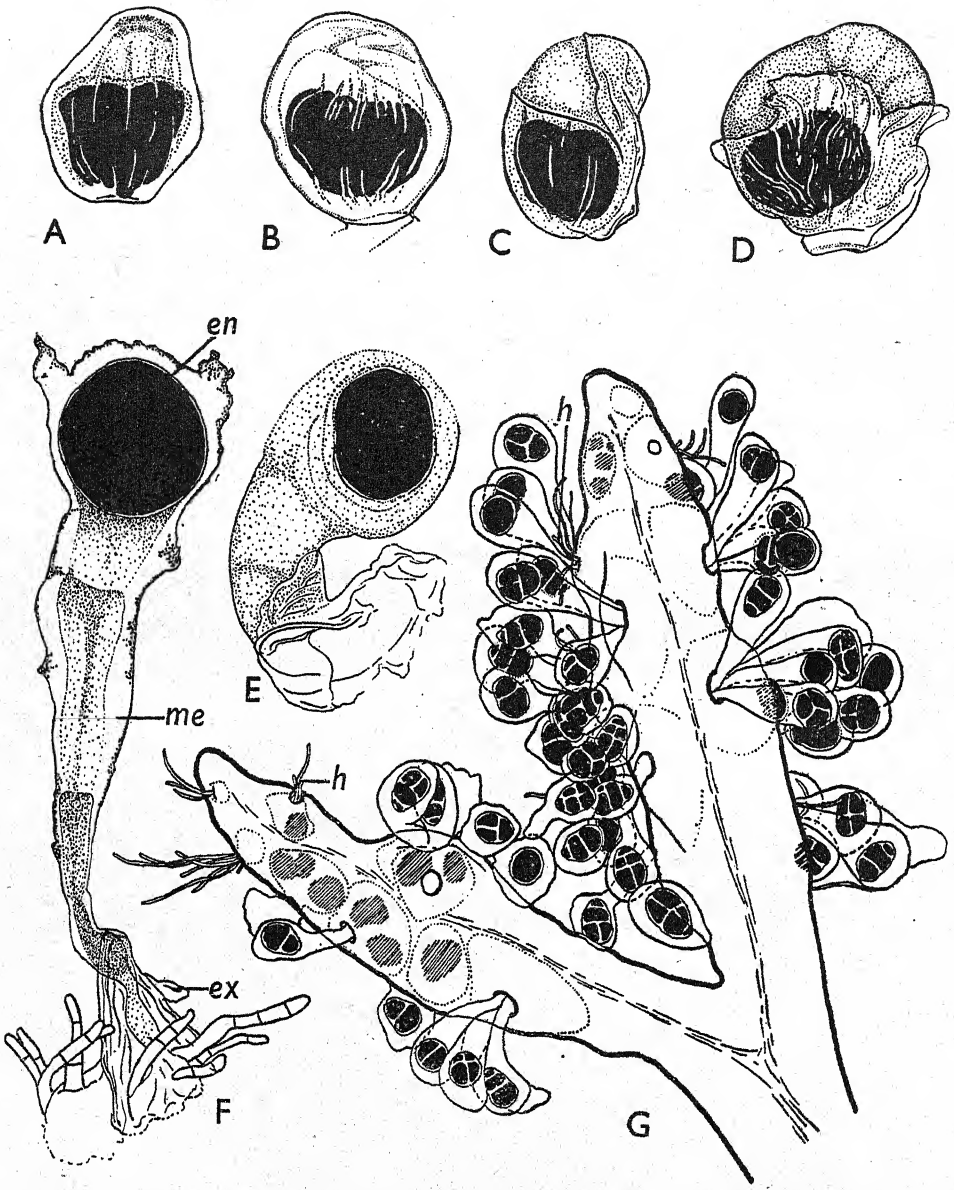

Fig. 2. *Cystoseira foeniculacea* (dissections). A. Oogonium with apical mucilage. B. Mucilage curving to one side. C. Exochiton ruptured, allowing mucilage to bulge out. D. Swelling and extension of mucilage into a strand. E. Short stalk holding contents free from exochiton. F. Completely extended stalk with ensheathed oosphere (*en*=endochiton, *me*=mesochiton, *ex*=exochiton; A—F × 150). G. Forked receptacle covered with ensheathed oospores (*h*=hairs; × 35).

the conceptacles are more likely than the receptacles to be periodic. The emerging masses, even though they differ from those of *Sargassum* and *Carpophyllum* in not including the whole complement of oospheres at one time, are nevertheless sufficiently dense to form surprisingly resistant, spawn-like films over the ostioles, and to enclose the receptacles almost completely in a mucilaginous coat.

Sauvageau (1909) made a few unillustrated observations on this kind of extrusion in the plexus of five species of *Cystoseira* already referred to, and described the fertilization of oospheres through a thick sheath. Unpublished observations by Dr E. M. Delf on *C. abrotanifolia* (from Athlit, Palestine), which I have been able to confirm, reveal sheaths indistinguishable in form and, I believe, in origin from those of *C. foeniculacea*. The same result is described for the oogonia of other submerged Sargassoids, but in many of the species investigated the state of the material apparently precluded observation of the complete process of stalk formation and emergence. The stages described for *Bifurcaria brassicaeformis* (Delf, 1935) tally closely with *Cystoseira foeniculacea*, and my conclusions on *Carpophyllum flexuosum* (Dawson, 1940) are compatible with the same explanation. Incomplete descriptions of stalks in species of *Sargassum* (Simons, 1906; Sauvageau, 1909; Tahara, 1909; Nienburg, 1910; Kuneida, 1928; Delf, 1935) and *Cystophyllum* (Delf, 1935) are also fully explicable on this basis. Hence it looks as though a uniform type of specialization of the mesochiton is widespread in this family.[1]

The accompanying table shows the record of observations on stalks. *Marginariella* is included, although its stalks develop by the unfolding of a hollow, pleated mesochitonous tube (Delf & Hyde, 1936; Delf, 1937) instead of by the lengthening of a solid slimy strand. This rarer method is hinted at in *Seirococcus axillaris*, where the few stages which I have seen suggest the symmetrical layering and 'earlike' folds of *Marginariella* rather than the lop-sided swelling of *Carpophyllum*, *Sargassum* and *Cystoseira*. The Australian genus *Seirococcus* like *Marginariella* belongs morphologically to the Fucaceae (Gruber, 1896; Schmidt, 1938). The division of the Fucales into the two families Fucaceae and Sargassaceae has a yet stronger claim to naturalness on the evidence of such a clear example of homoplasy as the formation of mesochitonous stalks in two distinct ways. The table given below also attempts to record the sexuality of the conceptacles concerned in producing stalked 'oogonia', which are not confined to unisexual types. As Kniep pointed out (1907), for example, in *Cystoseira*, monoecism is often retained in conditions favouring dioecism. In fact dioecism of the thallus can scarcely be regarded as reliably established in any species of *Sargassum* (Setchell, 1936). Records of it even in conceptacles are often inadequate since 'protandry' gives a temporary impression of segregation, e.g. in *Bifurcaria tuberculata* and *Marginariella Urvilleana* (Delf). It is possible that two tendencies are at work: one towards separation of oogonia and actively functional antheridia on distinct plants as in *Carpophyllum*, *Turbinaria*,

[1] In *Bifurcaria* (=*Pycnophycus*) *laevigata*, E. M. Laing shows that, after the establishment of four functional oospheres in each oogonium, local secretion of mucilage between the protoplast and the exochiton provides every oosphere with a slimy strand. This case is unique in that each stalk belongs to one oosphere of a set, instead of to the one survivor of eight or to a syncytium. The degree of correspondence between the two types of stalk remains to be seen.

and *Marginariella*; the other as in Sauvageau's five species of *Cystoseira* towards the reduction of spermatozoidal activity in a monoecious thallus.

Mesochitonous stalks in the Sargassaceae

Species	Observer	Date	Locality	Sexuality of conceptacles
Bifurcaria brassicaeformis	Delf	1935	S. Africa: Cape	m.+f.
B. laevigata?	Laing	1941	S. Africa: Cape	h.
Carpophyllum elongatum	Delf	1939	New Zealand	m.+f.
C. flexuosum	Dawson	1940	New Zealand	m.+f.
C. maschalocarpum	Delf	1939	New Zealand	m.+f.
C. scalare (=*Contarinia australis*)	Dawson	Unpub.	S. Africa: Cape	h.
C. longifolium (=*Anthophycus longifolius*)	Laing	Unpub.	S. Africa: Cape	—
Coccophora Langsdorfii	Tahara	1928	Japan	m.+f.
Cystophyllum crassipes	Inoh	1938	Japan	m.+f.
C. muricatum	Delf	1935	Australia	m.+f.
C. sisymbrioides	Tahara	1913	Japan	m.+f.
C. trinode	Delf	1935	Red Sea	m.+f.
Cystoseira abrotanifolia	Sauvageau	(1909) 1912	Mediterranean Sea	h.
	Delf	Unpub.	Mediterranean Sea	
C. canariensis	Sauvageau	(1909) 1912	Canary Is.	h
C. discors	Sauvageau	(1909) 1912	Mediterranean Sea	h.
C. foeniculacea	Sauvageau	(1909) 1912	Atlantic coast, France	h.
C. foeniculacea	Dawson	1941	Brittany	h.
C. myriophylloides	Sauvageau	1912	N.W. France	h.
**C. barbata*?	Dodel-Port	1885	Mediterranean Sea	h.
†*Marginariella Boryana*	Delf & Hyde	1936	New Zealand	m.+f.?
†*M. Urvilleana*	Delf	1937	New Zealand	h.
Sargassum enerve	Tahara	1909	Japan	m.+f.
		1913		
S. Filipendula	Simons	1906	N.E. America	h.
S. Filipendula forma *compactum*	Dawson	Unpub.	Atlantic drift, W.I.	h.
S. hemiphyllum	Inoh	1930	Japan	m.+f.
	Delf	1935		
S. Horneri	Tahara	1913	Japan	m.+f.
	Kuneida	1928		
	Okabe	1930		
S. incisifolium	Delf	1935	S. Africa	m.+f.
S. Kjellmanianum	Inoh	1930	Japan	m.+f.
S. lendigerum	Delf	1935	S. Africa: Natal	m.+f.
S. linifolium	Kniep	1907	Mediterranean Sea	h.
	Nienburg	1910	Adriatic	—
S. serratifolium	Inoh	1930	Japan	m.+f.
S. Thunbergi	Tahara	1929	Japan	m.+f.
	Inoh	1930		
S. vulgare var. *flavifolium*	Sauvageau	1909	Atlantic drift: Biscay	m.+f.
		1912		
Turbinaria filiformis	Inoh	1938	Japan	m.+f.
T. fusiformis	Tahara	1929	Japan	m.+f.
T. ornata	Inoh	1938	Japan	m.+f.

* Dodel-Port (1885) described liberated oogonial contents caught and held amongst the cryptostomatal hairs of *C. barbata*. This is the first record of *some* kind of adherence.

† Fucaceae.

h.=hermaphrodite, m.=male, f.=female.

Cytological maturation and embryogeny

The first hints of nuclear activity, suggestive of meiotic prophase (cf. Dawson, 1940), coincide with the beginning of the above changes in the oogonial wall. But extrusion appears to be achieved before the completion of the three nuclear divisions which produce the eight potential oosphere initials; for eight-nucleate protoplasts have only been found outside the conceptacles. The moment of cytological maturation thus contrasts with the similar stage in *Cystoseiras* with unattached oospheres like *C. osmundacea* (Gardner, 1910) and *C. barbata* Ag. (Nienburg, 1910), where the process is finished inside the conceptacles. Its postponement in *C. foeniculacea* is probably connected with the relatively autonomous gametophyte: the attached 'oocyte' (cf. Delf, 1939*b*). According to several descriptions (Sauvageau, Gardner, Nienberg) the seven supernumerary nuclei are cast out peripherally, leaving all their cytoplasm and the eighth product of meiosis to form one large, syncytial oosphere. The thin endochiton and thick mesochiton in Fig. 2 F surround what is presumably this aggregate.

Antheridia and oogonia are so close together and contemporaneous in development that, apart from possible self-incompatibility, fertilization would appear inevitable. Certainly germinating oospores are abundant (Fig. 2 G). The sporelings they produce are of the Sargassaceous type, in which the rhizoidal cell divides before the tuft of functional rhizoids protrudes. In this case a group of only four develops (Fig. 1 E) and the sporeling reaches a size of about 165μ long by 125μ wide before the dissolution of the sheath releases it.

Discussion

Cystoseira foeniculacea shows very clearly that specialization does not proceed in all directions at the same rate. For a Sargassaceous species its morphology is relatively simple when compared with *Sargassum* or *Turbinaria*. Anatomically it is retentive of the trichothallic method of growth which is considered fundamental to several evolutionary series in the Phaeophyceae. The linings of the sunken pits where this growth is localized retain their activity for a long time and yield a variety of products. Long hairs splay out of the conceptacles as well as out of the mouths of pure hair pits; and oogonia are formed intercalarily rather than direct from the lining cells of the conceptacles.

The close proximity of the antheridia and oogonia leads to the inference of self-fertilization, which may to some extent be obviated by the development of unisexual conceptacles, and reduction in the total number of antheridia. Dioecism is common in submerged Sargassoids, as is a mechanism for preventing the sinking of the heavy expelled reproductive cells. The agency of desiccation is inoperative in their liberation. The external factors responsible—varying hydrostatic pressure and changing illumination presumably—act upon a highly modified layer of the oogonial wall, which unfolds into a stalk continuous with a sheath round the whole protopast. Far from being simply a trailing strand of slime as some of the earliest

observers implied, this is an intricately specialized and resistant organ which develops uniformly in a number of widely dispersed Sargassaceous species both morphologically simple and complex. It provides great security by its secondary internal adhesion to the exochiton, and by its complete enclosure of the cell contents which it lifts out of the conceptacle. What I have for simplicity called the oogonium is probably a composite organ—a megasporangium producing a syncytial gametangium one of whose gametes develops after extrusion at the expense of the others (cf. Delf, 1939*b*; Dawson, 1940). On this interpretation the relatively well-established gametophytic individual, bounded by the endochiton, may be another primitive trait in *C. foeniculacea*, compared with the emergent Fucoids and with most other *Cystoseiras*, but it is impossible to distinguish between simple characteristics which are truly primitive and those which are reversionary.

SUMMARY

1. *Cystoseira foeniculacea* (L.) Grev. emend. Sauvageau from Brittany is a monoecious species. Its conceptacles retain trichothallic growth which results in the production of long centrally placed hairs like those of cryptostomata, as well as in the intercalary development of oogonia. Hitherto the derivation of an oogonium from a cell of a hair has only been recorded in *Notheia anomala* and *Durvillea potatorum*.

2. The middle layer of the oogonial wall produces specialized slime whose extension into a tough stalk has been initiated and followed experimentally. A table is given of the record of mesochitonous stalks in more than 30 widely separated species of the Sargassaceae. Mesochitonous stalks, developed slightly differently, occur in three members of the Fucaceae.

3. The single oosphere is only elaborated during or after extrusion. After fertilization it produces a typical Sargassaceous sporeling with four primary rhizoids.

4. The species shows a number of primitive features, probably correlated with the equable conditions in which it lives. The free gametophyte may or may not be primitive, but its attachment is highly adaptive.

It is a pleasure to record my gratitude to Dr E. M. Delf for helpful criticism and for assistance in preparing this paper for publication.

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CYTOLOGY OF *HIBISCUS TRIONUM* L.[1]

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(With 33 figures in the text)

The genus *Hibiscus* is closely related to *Gossypium*, both being included in the tribe Hibisceae. It has therefore been the subject of investigation by many cytologists, especially with a view to determining the chromosome number of its members. Very few species of *Hibiscus* have, however, been worked out in detail from a cytological point of view. The investigations carried out to date indicate that the members of the genus fall into several different series of chromosome numbers with different basic numbers (cf. Table 1). Indeed, the genus appears to match *Brassica* in the diversity of its basic chromosome numbers. It was hoped that a closer study of one species might give some indication of the nature of the changes which may have contributed to this diversity.

Material and method

For mitotic stages, root tips from seeds germinated on moist filter paper in Petri dishes at room temperature were employed. They were fixed in chromic formalin of the ratio 6:4 which was found to be well suited to the material. Both smear and section methods were followed. For smearing, the tips were fixed for 2½–3 hr., washed for ½ hr. and then stained by the treatment with Feulgen reagent. For microtome sections, the tips were fixed for 24 hr. and finally embedded in paraffin. Material for the study of the meiotic stages was collected on a number of warm sunny days, from plants grown in Regent's Park, London. Medium Fleming and Levitsky's chromic formalin were the most important fixatives used. Most of the material, however, was fixed in chromic formalin with a previous dip in pure chloroform instead of Carnoy. This procedure ensures fixation by the fixing fluid and not by the rapidly penetrating Carnoy's fluid. An exhaust pump was used to secure uniform fixation of buds by keeping them well immersed in the fluid. After 24 hr. in the fixative, the material was washed and prepared for embedding in paraffin. The presence of abundant mucilage in the tissue not only makes smearing difficult but also prevents the mother cells from adhering to the slide; consequently the smear method was abandoned after trial.

In this connexion I have to thank Prof. R. R. Gates, F.R.S., King's College, London, who suggested this problem and permitted me to use the material for this investigation out of his cultures at Regent's Park, London.

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Table 1

The genus *Hibiscus* includes about 200 species and cytological data are not yet available about most of them. As the following list shows, even the chromosome number has been determined only for very few.

Name of the species	*n*	*2n*	Source
H. cannabinus L.	—	36	Breslavetz (1934)*
H. esculentus L.	—	132	,,
H. trionum (=*africanus*)	—	56	Davie (1933)
H. coccineus Walt.	—	37–50	Kesseler (1932)
H. esculentus	59–60–61	—	Krenke†
H. militaris Cav.	19–20	—	Longley (1933)
H. Moscheutos L. (=*H. palustris* L.)	19–20	—	,,
H. Surattensis L.	36–39	—	Margadant†
H. Phoeniceus L.	11	—	Skovsted (1935)
H. Cannabinus	18	36	,,
H. Sabdariffa L.	36	72	,,
H. Gossypinus Thunb.	14	—	,,
H. trionum L.	28	56	,,
H. Parkeri Baker	—	34	,,
H. Solandra L'Herit.	17	34	,,
H. vitifolius	—	34	,,
H. roseus Thore	—	38	,,
H. Waimeae A. A. Heller	42	—	,,
H. tiliaceus	—	92	,,
H. Collinus Roxb.	—	92	,,
H. Rosa sinensis	—	92	,,
H. mutabilis L.	—	92	,,
H. Manihot L.	33	66	,,
H. Abelmoschus L.	36	72	,,
H. ficulneus L.	—	78	,,
H. Manihot	30	—	Teshima (1933)
H. esculentus L.	36	—	,,
H. Tricuspis Banks	40	—	Youngman (1931)
H. Rosa sinensis L.	72	—	,,
H. Tiliaceus L.	48	—	,,

* From Skovsted. † From Tischler.

Mitosis

The somatic chromosome number in *Hibiscus trionum* has been determined to be 28 (Fig. 1). Davie (1933) in *H. Africanus majus*, which name is a synonym for *H. trionum*, has recorded the somatic number to be 56, while Skovsted (1935) has also observed $2n=56$ in *H. trionum* L. However, Nakajima (1936) has counted the $2n$ number as 28 in the same species and suggested that Skovsted's material belonged to a tetraploid race of the same species.

In the present material, the somatic chromosomes are small, ranging from 2·2 to 5·5μ in length. Some of the chromosomes of the set can be distinguished easily from others by the presence of trabants; in favourable plates six chromosomes of the somatic set can be seen to have trabants. Two of these trabants are of comparatively large size, while the other four are relatively small (Fig. 2). The centromere constrictions of the chromosomes are approximately median, dividing each chromosome into two more or less equal arms. In some chromosomes, however, one arm appears to be rather longer than the other. There are no secondary constrictions on any of the chromosomes, apart from those separating the trabants from the main body of the chromosomes bearing them. The centromere appears

to be the last portion to undergo splitting and the first to separate during anaphase. The separating chromosomes are in general V and J-shaped. Just before the complete separation of the chromosomes when the sets of daughter chromosomes are moving to the poles, the trabants can easily be made out at the ends of the long arms bearing them (Fig. 5).

At prophase, the gradual condensation of chromatin and its organization into closely coiled threads inside the nucleus can be followed. The threads become progressively thicker and less coiled as prophase advances and a split becomes recognizable in well-differentiated portions. During these changes the nucleus enlarges, reaching its maximum size, while the nucleolus also becomes very large. Some of the chromosomes are attached to the nucleolus. The maximum number of such attachments, as can be made out in good Feulgen light green preparations, was found to be six when there was a single nucleolus in the nucleus (Fig. 4). When more than one nucleolus is present in the nucleus, each of them has one or more chromosomes attached, the total number being always six per nucleus (Fig. 3). At the point of attachment each thread was found to end in a small body, i.e. trabant, which is joined to the main body by a delicate achromatic connecting filament. In certain preparations this filament could be seen extending over the surface of the nucleolus.

An examination of the telophase nucleus shows the development of six nucleoli as independent bodies which later on fuse to form either one large nucleolus or several nucleoli varying in size and in number from two to five. The nucleoli take their origin from the chromosomes bearing trabants before the formation of the nuclear membrane. They were found to arise as small globular bodies in each group of late anaphase or early telophase chromosomes (Figs. 6, 7).

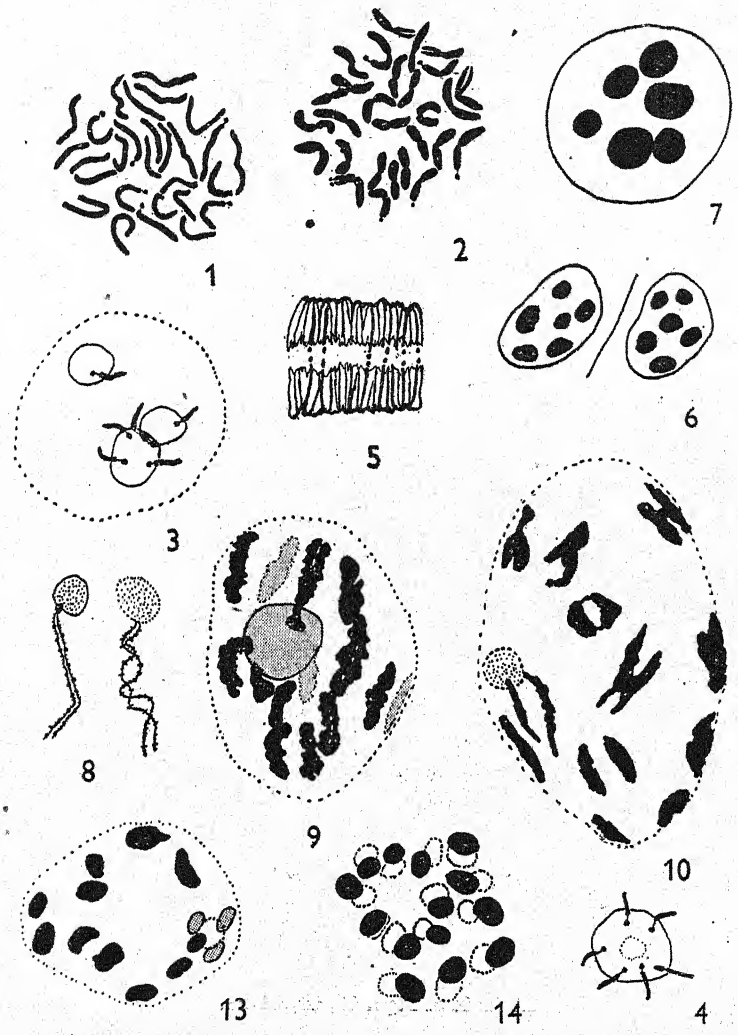
It is clear that the number and position of the nucleoli in the telophase nuclei depend upon the number and position of the chromosomes bearing trabants. The anaphase chromosome groups show symmetry in the relative position of their chromosomes, and the same is continued into late telophase and sometimes even to the resting stage. This accounts for the mirror symmetry observed in the position, number and even the size of the nucleoli of the daughter nuclei.

The nucleolus of the resting nucleus of both pollen mother as well as root-tip cells shows one or more inclusions, spherical, oval or in some cases crystalline in shape. In materials fixed in chromic formalin and treated with Feulgen reagent these inclusions are found to be spherical or oval in shape with a smooth outline and look like vacuoles, while in those stained with Newton's gentian violet they appear crystalline and highly refractive, sometimes with sharp angles. The vacuole-like appearance of these inclusions found in Feulgen preparations may probably be due to the action of hydrolysis.

MEIOSIS

The pollen mother cells are polygonal in shape and are closely packed in the locule, without interspaces. They are uniseriate in arrangement, sometimes biseriate, with the cells tending to alternate as seen in longitudinal section of the locule. The cytoplasm of the mother cell usually fills the cell and is densely granular and

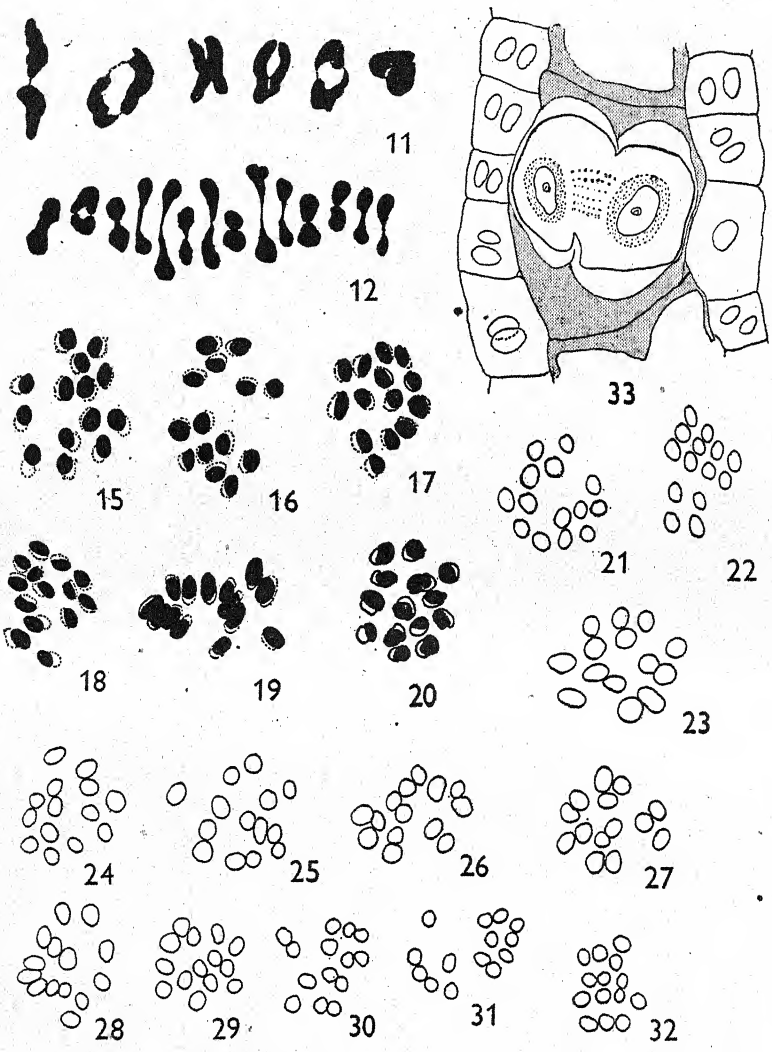
alveolar in appearance. It is not homogeneous, since there is a dense layer round the nucleus, the perinuclear zone, which is characteristic of Malvaceae and which becomes very distinct during meiosis. Isolated granules of material staining like chromatin with gentian violet, are found scattered in the cytoplasm. At no stage do the mother cells become completely separate from one another and float freely in the tapetal plasmodium.



Legends of Figs. 1–10, 13, 14.

Fig. 1. Chromosome complement from a cell of the root tip of *Hibiscus trionum*, showing six trabants. Fig. 2. Chromosomes from a root-tip cell showing the difference in the size of the trabants and constrictions. Figs. 3, 4. Nucleolar attachments from a somatic nucleus. Fig. 5. Trabants seen in the early anaphase chromosome sets. Figs. 6, 7. Number of nucleoli in the somatic nucleus. Fig. 6 shows the early telophase stage. Fig. 8. Pachytene threads showing chiasmata as well as relational coils. Figs. 9, 10. Stages in diplotene. Figs. 13, 14. Stages in the formation of bivalents.

The nucleus of the mother cell is large, usually spherical in shape with a more or less distinct nuclear membrane. It contains a densely staining nucleolus and less densely staining chromosome threads which are spread uniformly throughout



Legends of Figs. 11, 12, 15–33.

Fig. 11. Configuration of diplotene chromosomes. Fig. 12. Side view of metaphase I showing the chiasmata. Figs. 15–20. Polar views of metaphase I showing association of bivalents. Figs. 21–32. Polar views of metaphase II showing the association of univalents. Fig. 33. Cytokinesis in tetrad formation. The special wall around the dividing mother cell and its ingrowths cutting the cytoplasm of the latter into separate parts can be seen. × 1600.

All the figures were drawn with a Zeiss camera lucida, under a 2 mm. Zeiss oil-immersion objective N.A. 1·4 and compensating ocular × 18, giving a magnification of × 4000 at the table level with 120 mm. tube length. Fig. 33 was drawn using a compensating ocular × 6 giving a magnification of × 1600. Figures reduced to approximately one-half.

the cavity. As the prophase changes in the nucleus progress, the threads become more densely staining, with fine granules appearing scattered all over them and thus giving a beaded appearance. Some of the threads can be traced to the nucleolus, where they appear to end in a knob-like structure. At pachytene stage, the chromosomes are found to be thick and coiled, the granules or chromomeres increasing in size and staining property. The paired nature of the threads can be seen clearly (Fig. 8), while the chromomeres are unsplit. The two threads appear to twist around each other at intervals, and some at least of these overlaps can be seen to be chiasmata. Others are most probably relational coils of the paired chromosomes (Fig. 8). Subsequently, the threads become still thicker and shorter and the twists appear to decrease in number. Gradually the thick chromosomes come to have a rough and irregular outline or contour (Figs. 9, 10). The points of apparent contact between

Table 2

Cell plate no.	Solitary bivalents or univalents	Association of 2 bivalents or univalents	Association of 3 bivalents or univalents	Association of 4 bivalents or univalents	Association of 5 bivalents or univalents	Ref. to Figs.
			Metaphase I			
1	9	1	1	—	—	15
2	8	1	—	1	—	16
3	6	4	—	—	—	17
4	4	1	1	—	1	18
5	3	2	1	1	—	19
6	9	2	1	—	—	20
			Metaphase II			
1	10	2	—	—	—	21
2	8	—	2	—	—	22
3	4	5	—	—	—	23
4	8	3	—	—	—	24
5	6	2	—	1	—	25
6	1	3	1	1	—	26
7	1	5	1	—	—	27
8	6	—	1	—	1	28
9	7	2	1	—	—	29
10	4	5	—	—	—	30
11	5	3	1	—	—	31
12	7	2	1	—	—	32

the pairing threads are very much reduced in number. The reduction in number is probably to be attributed largely to the loss of relational coiling, the remainder being chiasmata. At a later stage the movement of the chiasmata consequent on the condensation of the chromosomes to form the bivalents takes place and the final position taken up by the chiasmata is shown in Figs. 11 and 12. During these stages, the nucleolus becomes gradually less chromatic and stains less deeply than ever (Fig. 10). As the bivalents take up a peripheral position inside the nucleus, their contour becomes smooth and they finally assume a short and oval shape (Fig. 13). By this time the nucleolus disappears and also the nuclear membrane. The perinuclear zone of the cytoplasm becomes very prominent, and it does not appear to take any part in the formation of the spindle. The spindle is intranuclear in origin, and its beginning can be made out even before the complete disappearance of the nuclear membrane.

The peripherally distributed bivalents now gradually move towards the centre of the cell, to take up their position on the equatorial plate of the spindle (Fig. 14). During this stage—the prometaphase—the chiasmata can be seen clearly and analysed (Figs. 11, 12). In one set of 14 bivalents, the total number of chiasmata found was twenty, thus giving the chiasma frequency of 1·4 per bivalent. Of these twenty, eight of the bivalents each had a single terminal chiasma; six others had two terminal chiasmata each. On the equatorial plate, the bivalents exhibit a certain amount of secondary association of the bivalents. Such association has been observed both in the 1st and 2nd divisions. A number of plates of both the divisions were studied and various types of association were observed (Table 2).

CYTOKINESIS IN TETRAD FORMATION

Cytokinesis in tetrad formation has not been studied in any member of Malvaceae in detail. The pollen mother cells which fill the cavity of the locule completely undergo certain changes when the meiotic prophase commences. The cytoplasm retracts from the walls adjacent to the tapetal cells, while it remains in contact with the walls separating the pollen mother cells. Between the cytoplasm and the mother-cell wall, a new layer of hyaline substance is deposited in close apposition to the wall. This layer has been called 'new wall' or 'special wall' by Beal (1928) and Gates (1924) in cotton and *Lathraea*. By the time the meiotic divisions are finished the special wall is complete. It is quite independent of that of the mother cell. Immediately after the second division, when the four daughter nuclei are still connected by spindles, small notches or indentations are noticeable at the periphery of the cytoplasm, approximately opposite the middles of the spindles and midway between the nuclei. These indentations, which gradually become more pronounced, are the beginning of the furrows leading to tetrad formation. At this stage the special wall is readily overlooked owing to its marked transparency. But in specially overstained preparations, simultaneously with the appearance of the cytoplasmic indentations, a wedged-shaped projection can be detected on the special wall opposite each indentation. These projections fit closely into the indentations. In the same measure as the projections extend inwards the indentations deepen. The inner edge of each projection is very slender and recognizable only in well-stained preparations (Fig. 33). Ultimately, the projections meet in the centre of the mother cell and the cytoplasm is divided into four parts, each surrounded by a special wall of varying thickness. As the special wall gradually becomes thicker, the mother cell wall breaks down and the tetrads lie in the cavity of the locule, still embedded in the hyaline matrix of the special wall.

The special wall which played a part in the formation and separation of the tetrads also appears to participate in the formation of the spore walls. As the spines and pittings develop on the outer spore wall, the transparency of the special wall increases. It appears to be used up in the formation of the spore membrane, since it ultimately disappears, so that the spores become free.

It is clear that tetrad formation in *Hibiscus trionum* is essentially effected by

furrowing of the cytoplasm as described by Farr (1916, 1918) in *Nicotiana* and *Magnolia*, but with one important difference. The furrowing of the cytoplasm is caused by the formation, from the special wall, at definite points, of wedge-shaped ingrowths which enlarge until they meet at the centre of the mother cell.

Discussion

It is well known that a great majority of seed-bearing plants are polyploids, usually allopolyploids. These allopolyploids, as is shown by the absence of multivalents, are the result either of crossing between species with rather different chromosomes or are not of very recent origin. The chromosome pairing in such allopolyploids takes place, according to Lawrence (1931), within the parental set and never or but rarely between them, i.e. the similar chromosomes from the different parental species are too unlike one another to pair and form quadrivalents or other multivalents. Their polyploid constitution is therefore not shown by the presence of multivalent associations; instead, the allopolyploids are characteristically functional diploids. Their polyploid nature can, then, be disclosed only in relatively indirect ways, namely, (*a*) that their chromosome number may be a multiple of that of related species, and (*b*) that similar bivalents in their set at meiosis may show secondary pairing at metaphase I or at metaphase II. It is in showing the polyploid constitution of the polyploid, though functionally diploid, species that secondary association is often a great help. Secondary association is thus an evidence of more remote affinity between homologous chromosomes than the one seen in the primary association, and can be used as an indication of the polyploid constitution of the plant.

The presence of secondary pairing and the absence of multivalents in *Hibiscus trionum* probably indicates that this is an ancient polyploid. From the types of secondary association as shown in Table 2, it is not possible to make any very definite deduction concerning the degree of polyploidy of *H. trionum*. However, the high frequency of groupings of three bivalents or of three univalents, at metaphases I or II respectively of meiosis, points to hexaploidy of at least a fair section of the basic chromosome complement. But the haploid chromosome number is 14 and this is not a whole multiple of three; consequently it is not improbable that the chromosome set is a secondarily balanced one, modified from a hexaploid structure.

De Mol (1928), as a result of his studies in diploid, triploid and aneuploid *Hyacinthus*, came to the conclusion that 'every somatic nucleus possesses the potency to develop a certain number of nucleoli, this number being genetically determined. The occurrence of two nucleoli in the diploids and three in the triploids gives occasion to surmise that originally a single nucleolus formed a unit with the haploid set of chromosomes.' Thus the number of nucleoli in the cells was considered a reliable guide to the polyploidy of the plant. This idea is reflected in the later work on species of *Allium* by Mensinkai (1939), *Oryza* and *Triticum* by Pathak (1940) and *Brassica* by Sikka (1940). The presence of six nucleoli in the somatic nucleus of *Hibiscus trionum* naturally suggests its hexaploid nature.

It must be pointed out in this connexion that the observations of McClintock

(1934) on a reciprocal translocation in maize, where the nucleolar organizer was broken into two by the action of X-rays and each part produced a separate nucleolus, are of significance. The fact that the two resulting nucleolar organizers continued to function separately shows the possibility that the number of nucleolar organizers may be increased as a result of structural changes. Haploid sets of chromosomes may thus come to produce two or more nucleoli, without there having been any increase in chromosome number.

The species of *Hibiscus* have been classified into seven groups by Hochreutiner; so far as the subdivisions can be tested, they appear to be coherent on a chromosome basis (Skovsted, 1935):

(1) The morphologically isolated *H. phoeniceus* has the chromosome number $n=11$; it is so far the lowest in the genus.

(2) There is a group of species forming members of the series with 6 as the basic chromosome number. The complete series being 18, 24, 36 and 72.

(3) There are species representing polyploids of seven, such as *H. Gossypinus* and *H. trionum*.

(4) Three species having $2n=34$ form a series.

(5) Two species with $2n=38$ form a separate series.

(6) There are a large number of species which are high polyploids like $n=40$, 48, 72 and 92.

(7) The *Abelmoschus* group with $n=33$, 36 and 65 stands separate.

No species of *Hibiscus* is known at present whose haploid chromosome number is 7, which number is assumed to be the basic number for the series to which *H. trionum* belongs, since it is the highest prime number of the series. The various groups of *Hibiscus* may be the outcome of various different secondary polyploid balances of the same original chromosome set. Different balances would have given morphological differences of a character that could be used by the systematists.

Davie (1933), on the other hand, recognized four cytologically independent types of evolutionary changes in the Malvaceae:

(1) Polyploidy arising directly from the ancestral type with a basic number 7 and culminating in a stable hexaploid type, probably allohexaploid, having arisen by duplication of the chromosomes in the triploid, resulting from a cross between a diploid and a tetraploid.

(2) Origin of *Gossypium*, in which is involved tetraploidy on the basis of 7 followed by fusion of two pairs into one pair, thus giving $n=13$ as the fundamental number of the genus.

(3) Hexaploidy arising as in the first type and by subsequent structural changes (chromosome fusion in this case).

(4) Octoploidy arising via tetraploidy from ancestral diploids.

He considers *H. africanus majus* ($2n=56$) which is supposed to be synonymous with *H. trionum* as an octoploid arising via tetraploidy from an ancestral diploid. The present investigation has shown that *H. trionum*, with $2n=28$, is not an octoploid.

Thus the present material appears to be ancestrally a polyploid as shown by the secondary pairing and functionally a diploid as judged by the behaviour of the chromosomes during meiosis. The presence of six nucleoli in the somatic nucleus can be explained as due to polyploidy or structural changes or a combination of the two.

Summary

1. The chromosome number of *Hibiscus trionum* is 28. There are 14 bivalents at meiosis.

2. Six of the somatic chromosomes bear trabants, two large and four relatively small.

3. The telophase nucleus possesses six nucleoli.

4. In prophase, six nucleolar attachments are observed.

5. During meiosis, secondary association of bivalents at metaphase I and of univalents at metaphase II shows a high percentage of groupings into threes.

6. It is concluded that *H. trionum* is a secondarily balanced polyploid, partly tetraploid and partly hexaploid.

My thanks are due to Dr D. G. Catcheside, D.Sc., Cambridge University, for valuable suggestions and criticism during the preparation of this paper and to Prof. F. E. Fritsch, F.R.S., for his continued interest and help during the investigation.

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REVIEWS

Die Klimaverhältnisse des Albisgebietes. By W. Lüdi and B. Stüssi. 9×6 in. Pp. 69, 9 text-figs. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich, Heft 18. Bern: Hans Hüber, 1941. Price 4.20 Swiss frs.

This publication records an analysis of detailed meteorological observations taken in a small area south of Zürich, including the mountain ridge of the Albis and the shores of the lake of Zürich. The sites all lie within the region of uniform *Fagus-Abies* forest, and at altitudes between 460 and 910 m. The measurements included precipitation, daily extremes of temperature, and in two places sunshine duration.

The authors bring out clearly how dependent climate is upon topography. The ridge of the Albis causes precipitation from the south-west winds, and there is strip distribution of summer rain in accord with the regular paths of thunderstorms. The varying altitude of the horizon in mountain country naturally affects sunshine duration records, but other perhaps less obvious effects are the high summer sunshine values on the crests which lie above the valley mists, and the low winter values caused there by the cloudiness. The mean maximum-minimum daily temperatures show the natural decrease with altitude, but the stations concerned show the influence of other factors also. Thus Lake Zürich has a modifying and regularizing influence on climate, and the high-lying stations show a limited temperature range, with low maxima and high minima, the latter due to the drainage away from them of cold air. Enclosed valleys show the opposite effect.

The importance to the ecologist of recognizing such manifold complexity and variability of climatic factors in mountain regions is apparent at once.

The authors conclude that the climate for the region as a whole has a strongly oceanic character, with high precipitation, moderate mean temperature and small range of extremes. They do, however, point out a range of relative oceanicity and continentality within the area, Zürich itself having the most continental climate.

Having got thus far in useful analysis, the authors unexpectedly throw all their data into the melting pot of another 'pluvio-thermic' formula, by which their nine stations can be arranged in order of 'pluvio-thermic quotients', which are said to indicate the range from oceanicity towards continentality. The formula seeks apparently 'den Klimacharakter klar und knapp zu fassen', but though the authors prefer their own formula[1] to those of Gams or Emberger, it hardly seems to get one any further in that analysis of the vegetation which such studies are meant to serve. It is from the detailed analysis of the local climates that one must proceed to consider the mechanisms by which species and communities are favoured or excluded. It is precisely the exact values of frost occurrences, temperature range and duration, sunshine hours and their distribution, etc., which must be known if we are to find experimentally how any one species responds critically to climate. One is aware, for instance, that the beech has its oceanic limits somewhere in southern or south-eastern Britain, and it appears that the summer sunshine values are often too low to cause flower-initial formation, and that late spring frosts destroy the stigmas and lead to a failure of the mast crop. In bringing down analysis of the operation of climatic effects in this way to the physiology and biology of the plants themselves, the 'pluvio-thermic' quotient can only be a very preliminary step. The range of these quotient values certainly gives a 'short-hand' notion of the range of climatic type within one community, but the importance of such ratios is easily overvalued, and the main task of detailed correlation with vegetational distribution is still going to depend on the detailed climatic analysis which the greater part of this work exemplifies.

H. GODWIN

[1] $$Q = \frac{1000\,P}{(TM \quad Tm)\quad (TM \quad Tm)},$$

where TM = mean maxima; Tm = mean minima in absolute temperatures, and P = mean total yearly precipitation in mm.

Botany of the Canadian Eastern Arctic. Part I. *Pteridophyta and Spermatophyta.* National Museum of Canada, Bulletin No. 92, 1940. By NICHOLAS POLUNIN. Pp. 408, maps and plates. Price $1.

This is the first of a series of four floristic and ecological reports. The area covered comprises the most northerly parts of Labrador and Quebec, the north-west coast of Hudson Bay, Melville Peninsula, and the islands of the eastern half of the Arctic Archipelago—in all about 420,000 square miles of land which, for purposes of giving ranges, is conveniently divided into ten major divisions. A brief account is given of the size and position of every one of these together with the names and position, in terms of latitude and longitude, of all the important botanical localities. A concise summary of the history of botanical exploration in the area, with a list of collectors and dates and localities of their collections, follows. The introductory part concludes with an outline of the author's special methods of treatment of his subject matter, and finally with acknowledgements.

The main part of the work consists of an enumeration of the species of vascular plants of the Canadian Eastern Arctic, with notes on their taxonomy, distribution, and occurrence and citation of specimens, many of which are of the author's own collecting. Four new species and a number of new varieties and forms are described, and most of them figured, but the publication is not intended to be a descriptive flora. The order and nomenclature followed are, in general, those of the Gray Herbarium of Harvard University. A summary tabulation of the species and of their distribution within the Canadian Eastern Arctic is given. There are also a valuable alphabetical list of bibliographical references of over eleven pages, an index, and a detached, folded map.

In this first part of the planned series there is no general review of the floristic or phytogeographical conclusions, and, in view of the publication of the other parts, it would be unfair to anticipate the possible findings of the author. The summary figures, given on p. 384, are, however, sufficiently striking to call for comment. A total of 37 families (3 Pteridophyta, 34 Spermatophyta) and 297 species (11 Pteridophyta, 286 Spermatophyta) constitute the known vascular flora of this enormous region—a simple expression of its striking floristic poverty, even though the author rightly describes himself as a 'lumper' rather than a 'splitter'. The largest families are Gramineae (40 species), Cyperaceae (38 species), Compositae (32 species), and Caryophyllaceae (24 species). These four families account for nearly half the known Spermatophyta. The numbers of species in the remaining families may be grouped as follows:

18 to 10 species per family	...	9 families
5 to 2 species per family	...	8 families
1 species per family	...	13 families

The number of Leguminosae (10 species) seems at first sight rather large for an Arctic area, but seven species belong to the one genus *Oxytropis*. The floristic poverty is somewhat greater than even the above figures indicate since a fair number of the species have a very local range within the area, about 46, for example, being recorded from only one of the ten divisions and often from only one locality within the division. Thus *Zostera marina* L. is recorded from only one locality on the west coast of Hudson Bay (it is known also from James Bay in the extreme south); *Hierochloe odorata* (L.), Wahlenb. only from North Labrador; *Carex capitata* L. only from South Baffin; *Lychnis alpina* L. only from North Labrador; *Sagina nodosa* (L.) Fenzl; and so on.

The author has evidently taken a great deal of trouble to straighten out the nomenclature and taxonomy of the species and paramorphs (intraspecific variants) with which he is concerned. Future students of the Arctic flora will be greatly indebted to him for the numerous facts he clearly states and especially for those based on first-hand observations in the field. He admits from time to time that his conclusions are more or less tentative but this is due either to the lack of sufficient data (material, wide field studies, or cytogenetical investigation) or to the material itself being of such a nature that the form of taxonomic expression and even the actual classification itself is largely a matter of immediate scientific convenience—more than one taxonomic formulation being valid. The different degrees of

polymorphism of the accepted species opens up very large questions on which one would have welcomed the author's own conclusions. Hybridization is recorded, at least as probable, between *Poa arctica* R.Br. and other *Poa* species, between several species of *Salix*, between *Lychnis furcata* (Raf.) Fernald and *L. apetala* L., and between species of *Potentilla*. Again, a rather large number of species are found to be very variable without the probability of interspecific hybridization, as, for example: *Arctagrostis latifolia* (R.Br.) Griseb., *Deschampsia caespitosa* (L.) P. Beauv., *Trisetum spicatum* (L.) Richt., *Catabrosa algida* (Soland.) Fries, *Poa glauca* M. Vahl, *P. pratensis* L., *P. alpina* L., *Festuca rubra* L., *Eriophorum angustifolium* Roth, *Carex Bigelowii* Torrey ex Schwein., *C. aquatilis* Wahlenb., *C. saxatilis* L., *Salix reticulata* L., *S. planifolia* (agg.) Pursh, *Betula glandulosa* Michx., *Cerastium alpinum* s.l. L., *Stellaria longipes* Goldie, *Cochlearia officinalis* s.l. L., *Draba alpina* s.l. L., *D. glabella* Pursh, *Braya purpurascens* (R. Br.) Bunge, *Saxifraga cernua* L., *S. caespitosa* L., species of *Potentilla*, *Castilleja pallida* s.l. (L.) Spreng., *Campanula rotundifolia* s.l. L., and *Arnica alpina* s.l. (L.) Olin.

One is tempted, perhaps, to suggest that the high degree of intraspecific variability is in some way a compensation for the low number of species relative to the size of the area. A rather more likely explanation is that the low number of species has allowed time for Dr Polunin and other botanists specializing on Arctic floras to study them more fully and to find space for the publication of their results. The reviewer's opinion is that intraspecific variability is the rule and not the exception for all floras and that Arctic floras in this respect but furnish one of the numerous clear examples of the rule.

That binomial designation in the form of 'species' covers a hotch-potch is very clearly brought out in this study. Thus we have such remarks as the following: *Juncus arcticus* Willd., 'perhaps a "reduced northern phase" of the extremely variable *J. balticus*'; *Oxyria digyna* (L.) Hill, 'This seems to be one of our few really "good" species'; *Cerastium alpinum* s.l. L., 'This is a *typus polymorphus* of the worst order'; *Cerastium Beeringianum* Cham. et Schl., 'I have used it [this species] as a sort of rubbish heap. . . '; *Cerastium Regelii* Ostenfeld, 'Now that this species has at last "caught on", it is being recognized from many places where it probably does not occur'; *Eutrema Edwardsii* R.Br., 'This species appears to go unchanged around the top of the world—unchanged, that is, in any feature of taxonomic importance'; *Sibbaldia procumbens* L., 'This seems to be an unusually good monomorph'; *Castilleja pallida* s.l. (L.) Spreng, 'Taken in the broad sense this is an atrocious *typus polymorphus*; treated otherwise it becomes a whole series of supposed species and subspecies which, at least to those who like myself are unable to understand them, appear to intergrade so freely as to be in most cases quite untenable as separate taxonomic units.'

These extracts, taken from their contexts, in no way reflect disadvantageously on the author or his work from which they are taken. They merely show how very tentative are many taxonomic conclusions when these have to be expressed in terms of species and suggest, moreover, that there is increasing need for an overhaul of taxonomic methodology. It may well be that experiment and cytogenetical investigation will resolve some of the problems and lead to a more certain taxonomy, though the dispute regarding the best status to be awarded to *Empetrum hermaphroditum* or to the possible divisions of *Cochlearia officinalis* suggests that something more than merely further facts is needed. The very great practical value of the species as the main taxonomic unit (a value greater perhaps outside than within pure taxonomy) makes it exceedingly difficult to propose any sound alternative scheme of wide general applicability but this should not blind biologists to the limitations of the orthodox scheme. Dr Polunin's work has the very great merit of giving the data and then stating, in no dogmatic form, the conclusion he has himself reached, leaving it to the reader to agree if he will or to make a better job of it if he can.

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PRINTED IN GREAT BRITAIN BY W. LEWIS, M.A., AT THE UNIVERSITY PRESS, CAMBRIDGE